



Kongeriget Danmark

Patent application No.: PA 2003 00827
Date of filing: 04 June 2003
Applicant:
(Name and address) Novo Nordisk A/S
Novo Allé
DK-2880 Bagsværd
Denmark

Title: Safe chemical uncouplers for the treatment of obesity

IPC: -

This is to certify that the attached documents are exact copies of the above mentioned patent application as originally filed.



Patent- og Varemærkestyrelsen
Økonomi- og Erhvervsministeriet

30 October 2003

Helle Schackinger Olesen
Helle Schackinger Olesen



PATENT- OG VAREMÆRKESTYRELSEN

04 JUNI 2003

SAFE CHEMICAL UNCOUPLERS FOR THE TREATMENT OF OBESITY

Modtaget

FIELD OF THE INVENTION

This invention relates to chemical uncouplers with a broader safety window making the use of them in treating obesity and, consequently, in the treatment of obesity related diseases and conditions such as atherosclerosis, hypertension, diabetes, especially type 2 diabetes (NIDDM (non-insulin dependent diabetes mellitus)), impaired glucose tolerance, dyslipidemia, coronary heart disease, gallbladder disease, osteoarthritis and various types of cancer such as endometrial, breast, prostate and colon cancers and the risk for premature death as well as other conditions, such as diseases and disorders, which conditions are improved by an increase in mitochondrial respiration, more attractive.

BACKGROUND OF THE INVENTION

Obesity is a well-known risk factor for the development of many very common diseases such as atherosclerosis, hypertension, type 2 diabetes (non-insulin dependent diabetes mellitus (NIDDM)), dyslipidemia, coronary heart disease, and osteoarthritis and various malignancies. It also causes considerable problems through reduced motility and decreased quality of life. The incidence of obese people and thereby also these diseases is increasing throughout the entire industrialised world.

The term obesity implies an excess of adipose tissue. In this context obesity is best viewed as any degree of excess adiposity that imparts a health risk. The cut off between normal and obese individuals can only be approximated, but the health risk imparted by the obesity is probably a continuum with increasing adiposity. In the context of the present invention, individuals with a body mass index (BMI = body weight in kilograms divided by the square of the height in meters) above 25 are to be regarded as obese

Even mild obesity increases the risk for premature death and conditions such as diabetes, dyslipidemia, hypertension, atherosclerosis, gallbladder disease and certain types of cancer. In the industrialised western world the prevalence of obesity has increased significantly in the past few decades. Because of the high prevalence of obesity and its health consequences, its prevention and treatment should be a high public health priority.

Except for exercise, diet and food restriction, which is not feasible for a vast number of patients, no convincing treatment for reducing body weight effectively and acceptably currently exist. However, not only in view of the considerable problems directly related to obesity as described above, but also due to the important effect of obesity as a risk factor in serious

and even mortal and common diseases, it is important to find pharmaceutical compounds which are useful in prevention and/or treatment of obesity.

When energy intake exceeds expenditure, the excess calories are stored predominately in adipose tissue, and if this net positive balance is prolonged, obesity results, i.e.

there are two components to weight balance, and an abnormality on either side (intake or expenditure) can lead to obesity. This process may be counteracted by increasing the energy expenditure (for instance via exercise) or decreasing the energy intake (for instance by dieting). Pharmacological treatment available up to date only consists of Sibutramine (acting via serotonergic mechanisms, Abbott) and Orlistat (reducing fat uptake from the gut, Roche Pharm) neither reducing body weight effectively nor acceptably. There is therefore a need for pharmaceutical compounds which may be useful in prevention and/or treatment of obesity, for instance by increasing the energy expenditure or decreasing the energy intake.

One way of increasing energy expenditure is by increasing the metabolic rate. Oxidative phosphorylation in mitochondria, the energy from glucose metabolism and free fatty acids oxidation is used to drive the phosphorylation of ADP to ATP. When NADH and FADH_2 formed in the TCA cycle are oxidised back to NAD^+ and FAD respectively, protons are pumped out of the mitochondrial matrix. The resulting pH gradient (matrix pH=8 and outside pH=7) and potential (~ -170 mV, inside negative) across the inner mitochondrial membrane constitute the electrochemical proton gradient. As the effect of a one-unit pH difference corresponds to a potential of 61.5mV, the electrochemical proton gradient exerts a proton-motive force of roughly -230 mV, which is the driving force for the mitochondrial ATP synthesis.

When the ATP consumption thus increases, the cells respond by increasing the ATP synthesis and consequently the inward flux of protons through the ATP synthase, the enzyme responsible for ATP synthesis and thereby the metabolic rate is increased. Chemical uncouplers are compounds, which can transport protons across membranes, and when protons are transported across the inner mitochondrial membrane, the ATP synthase is bypassed. At the (alkaline) matrix side the proton is released and the deprotonated uncoupler returns to the inter-membrane space where it picks up another proton. The cycling of the uncoupler (or ATP synthesis) and the resulting proton transport leads to an increased outward pumping of protons through an increased oxidation of NADH and FADH_2 by the respiration chain. The NADH concentration in the matrix will consequently drop. Since NADH feed-back inhibits three steps in the TCA cycle (NADH is the main regulator of the TCA cycle), the flux through the TCA cycle will increase. Hence, the metabolic rate will increase.

Compounds, such as chemical uncouplers, which act by increasing the metabolic rate may thus be useful for treating obesity, but also for treating other conditions such as atherosclerosis, hypertension, diabetes, especially type 2 diabetes (NIDDM (non-insulin dependent diabetes mellitus)), dyslipidemia, coronary heart disease, gallbladder disease, osteoarthritis and various types of cancer such as endometrial, breast, prostate and colon cancers and the risk for premature death as well as other conditions, such as diseases and disorders, which conditions are improved by a reduced mitochondrial potential.

Furthermore, chemical uncouplers may reduce reactive oxygen species (ROS) that are assumed (De Grey et al, Eur J. Biochem 269, 1995 ff (2002)) to be involved in the aging process, in damage of heart tissue as well as neuronal tissue. It is therefore also possible that conditions affected by ROS may be reversed or halted by intervention by chemical uncouplers.

The best known chemical uncoupler is 2,4-dinitrophenol (DNP), which has been shown to increase energy expenditure in humans as well as animals. The side effects at higher doses include increased perspiration, vasodilatation, skin rashes, cataracts, neuritis and death! Two fatalities amongst the first 100.000 persons treated with DNP, and the fact that the lowest dose, which could be lethal, was only twice the average dose giving a desired 50% increase in basal metabolic rate giving a very narrow safety window combined with other factors led to the removal of DNP from the market. Since then nobody have attempted to develop or market uncouplers for the treatment of obesity.

DNP is the best known chemical uncoupler; but many other compounds are known to induce uncoupling. DNP derivatives such as 4,6-dinitro-*o*-cresol (Victoria Yellow) and 2,4-dinitro-1-naphthol (Martius Yellow) as well as structurally unrelated compounds such as 2,6-di-*t*-butyl-4-(2',2'-dicyanovinyl)phenol (SF6847) (also known as 2-(3,5-di-*tert*-butyl-4-hydroxybenzylidene)-malononitrile), carbonylcyanide *m*-chlorophenylhydrazone (CCCP) and carbonylcyanide *p*-trifluoromethoxy-phenylhydrazone (FCCP) (Miyoshi H et al. Quantitative relationship between protonophoric and uncoupling activities of analogs of SF6847 (2,6-di-*t*-butyl-4-(2',2'-dicyanovinyl)phenol), *Biochimica et Biophysica Acta* 891, 293-299 (1987)) are uncouplers.

Another class of chemical uncouplers is the salicylanilides of which S-13 is the most potent compound discovered so far (Terada H et al. Structural Requirements of Salicylanilides for Uncoupling Activity in Mitochondria Quantitative Analysis of Structure- Uncoupling Relationships, *Biochimica et Biophysica Acta* 936, 504-512 (1988)).

Goto K et al, Chem. Pharm. Bull. 44(3), 547-551 (1996) describes diethyl 4-[(4-bromo-2-cyanophenyl)carbamoyl]-benzylphosphonate for use as an LPL activator.

T. Shimokawa et al, Drug Development Research 51(1), 43-48 (2000) describes 5-chloro-N-(2-chloro-4-nitrophenyl)-2-hydroxy-3-methylbenzamide for use as a glucose uptake stimulator.

5 WO00/06143 to Texas Pharmaceuticals Inc. relates to a method for inducing intracellular hyperthermia comprising a step of administering a mitochondrial uncoupling agent, such as 2,4-dinitrophenol.

US 4,673,691 to Bachynsky relates to the use of 2,4-dinitrophenol for treating obesity.

SUMMARY OF THE INVENTION

10 The present invention provides the use of chemical uncouplers for enhancing mitochondrial respiration, which chemical uncouplers have an acceptably broad safety window making them useful for treating conditions benefiting from an enhancement of mitochondrial respiration, such as obesity, atherosclerosis, hypertension, diabetes, especially type 2 diabetes (NIDDM (non-insulin dependent diabetes mellitus)), dyslipidemia, coronary heart disease,
15 gallbladder disease, osteoarthritis and various types of cancer such as endometrial, breast, prostate and colon cancers and the risk for premature death as well as other conditions, such as diseases and disorders, which conditions are improved by a reduced mitochondrial potential due to a saturation of the uncoupling and thereby the increase of the metabolism.

DESCRIPTION OF THE FIGURES

20 Figure 1 shows the curve for the stimulation of glucose utilisation caused by SF6847 calculated as a percentage of the stimulation of glucose utilisation caused by DNP according to Assay (I) and how the curve will look for a partial compound according to the present invention. E_{max} is the highest level of stimulation that can be achieved by use if the test compound measured in percentages of the highest level of stimulation achieved by DNP. M is the
25 molar concentration of test compound.

Figure 2 shows how the calculation of EC_{50} in Assay (I) is performed, exemplified by DNP and a test compound according to the present invention. E_{max} is the highest stimulation of glucose utilisation achieved by the test compound and EC_{50} is the concentration of test compound that gives a 50% stimulation.

30 Figure 3 shows how the glucose utilisation depends on the concentration of the compound for 1) a compound with a decline in efficacy at concentrations slightly above the concentration at which E_{max} is achieved (CE_{max}) and for 2) a compound with no decline in

glucose utilisation at concentrations ranging from the concentration at which E_{max} is achieved (CE_{max}). See also Table 1.

DEFINITIONS

The term "alkyl" as used herein, alone or in combination, refers to a straight or branched chain saturated monovalent hydrocarbon radical having from one to twelve carbon atoms, also denoted as C_{1-12} -alkyl. Typical alkyl groups are alkyl groups with from one to eight or from one to six carbon atoms, also denoted as C_{1-8} -alkyl and C_{1-6} -alkyl respectively. Typical C_{1-6} -alkyl groups include, but are not limited to e.g. methyl, ethyl, n-propyl, isopropyl, n-butyl, sec-butyl, isobutyl, tert-butyl, n-pentyl, 2-methylbutyl, 3-methylbutyl, 4-methylpentyl, n-pentyl, n-hexyl, 1,1-dimethylpropyl, 1,2-dimethylpropyl, 2,2-dimethylpropyl (neopentyl), 1,2,2-trimethylpropyl and the like, while typical C_{1-8} -alkyl groups include the same groups as well as alkyl groups having seven or eight carbon atoms, such as heptyl, octyl, 2,2-dimethylhexyl and the like. The term " C_{1-6} -alkyl" as used herein also includes secondary C_{3-6} -alkyl and tertiary C_{4-6} -alkyl. The term " C_{1-8} -alkyl" as used herein also includes secondary C_{3-8} -alkyl and tertiary C_{4-8} -alkyl. The term " C_{1-12} -alkyl" as used herein also includes secondary C_{3-12} -alkyl and tertiary C_{4-12} -alkyl.

The term "alkenylene" as used herein, alone or in combination, refers to a straight or branched chain divalent hydrocarbon radical having from two to six carbon atoms and at least one carbon-carbon double bond, for example $C_{(3-6)}$ -alkenylene. Typical $C_{(3-6)}$ -alkenylene groups include, but are not limited to, propene-1,3-diyl, 1,3 butadiene-1,4-diyl, and the like. The term "conjugated alkenylene" as used herein, alone or in combination, refers to an alkenylene having consecutive double bonds, such as for instance 1,3 butadiene-1,4-diyl.

The term "cycloalkyl" as used herein, alone or in combination, refers to a non-aromatic carbocyclic monovalent hydrocarbon radical having from three to twelve carbon atoms, and optionally with one or more degrees of unsaturation, for example C_{3-6} -cycloalkyl. Such a ring may be optionally fused to one or more benzene rings or to one or more of other cycloalkyl ring(s). Typical C_{3-6} -cycloalkyl groups include, but are not limited to, cyclopropyl, cyclobutyl, cyclopentyl, cyclohexyl, cycloheptyl, cyclooctyl and the like.

The term "aryl" as used herein, alone or in combination, refers to a carbocyclic aromatic ring radical or to a fused aromatic ring system radical. Aryl is also intended to include the partially hydrogenated derivatives of the carbocyclic systems. Typical aryl groups include, but are not limited to, for example phenyl, biphenyl, naphthyl, and the like.

The term "heteroaryl", as used herein, alone or in combination, refers to an aromatic ring radical with for instance 5 to 7 member atoms, or to a fused aromatic ring system radical with for instance from 7 to 18 member atoms, containing one or more heteroatoms selected from nitrogen, oxygen, or sulfur heteroatoms, wherein N-oxides and sulfur monoxides and sulfur dioxides are permissible heteroaromatic substitutions; such as e.g. furanyl, thienyl, thiophenyl, pyrrolyl, imidazolyl, pyrazolyl, triazolyl, tetrazolyl, thiazolyl, oxazolyl, isoxazolyl, oxadiazolyl, thiadiazolyl, isothiazolyl, pyridinyl, pyridazinyl, pyrazinyl, pyrimidinyl, quinolinyl, isoquinolinyl, benzofuranyl, benzothiophenyl, indolyl, and indazolyl, and the like. Heteroaryl is also intended to include the partially hydrogenated derivatives of the heterocyclic systems enumerated below.

Examples of "aryl" and "heteroaryl" includes, but are not limited to phenyl, biphenyl, indenyl, fluorene, naphthyl (1-naphthyl, 2-naphthyl), anthracenyl (1-anthracenyl, 2-anthracenyl, 3-anthracenyl), thienyl (2-thienyl, 3-thienyl), furanyl (2-furanyl, 3-furanyl), indolyl, oxadiazolyl, isoxazolyl, thiadiazolyl, oxatriazolyl, thiatriazolyl, quinazolinyl, fluorenyl, xanthenyl, isoindanyl, benzhydryl, acridinyl, thiazolyl, pyrrolyl (1-pyrrolyl, 2-pyrrolyl, 3-pyrrolyl), pyrazolyl (1-pyrazolyl, 3-pyrazolyl, 4-pyrazolyl, 5-pyrazolyl), imidazolyl (1-imidazolyl, 2-imidazolyl, 4-imidazolyl, 5-imidazolyl), triazolyl (1,2,3-triazol-1-yl, 1,2,3-triazol-4-yl, 1,2,3-triazol-5-yl, 1,2,4-triazol-3-yl, 1,2,4-triazol-5-yl), oxazolyl (2-oxazolyl, 4-oxazolyl, 5-oxazolyl), isoxazolyl (isoxazo-3-yl, isoxazo-4-yl, isoxazo-5-yl), isothiazolyl (isothiazo-3-yl, isothiazo-4-yl, isothiazo-5-yl), thiazolyl (2-thiazolyl, 4-thiazolyl, 5-thiazolyl), pyridinyl (2-pyridinyl, 3-pyridinyl, 4-pyridinyl), pyrimidinyl (2-pyrimidinyl, 4-pyrimidinyl, 5-pyrimidinyl, 6-pyrimidinyl), pyrazinyl, pyridazinyl (3-pyridazinyl, 4-pyridazinyl, 5-pyridazinyl), quinolinyl (2-quinolinyl, 3-quinolinyl, 4-quinolinyl, 5-quinolinyl, 6-quinolinyl, 7-quinolinyl, 8-quinolinyl), isoquinolinyl (1-isoquinolinyl, 3-isoquinolinyl, 4-isoquinolinyl, 5-isoquinolinyl, 6-isoquinolinyl, 7-isoquinolinyl, 8-isoquinolinyl), benzo[b]furanyl (2-benzo[b]furanyl, 3-benzo[b]furanyl, 4-benzo[b]furanyl, 5-benzo[b]furanyl, 6-benzo[b]furanyl, 7-benzo[b]furanyl), 2,3-dihydrobenzo[b]furanyl (2-(2,3-dihydro-benzo[b]furanyl), 3-(2,3-dihydro-benzo[b]furanyl), 4-(2,3-dihydro-benzo[b]furanyl), 5-(2,3-dihydro-benzo[b]furanyl), 6-(2,3-dihydro-benzob]furanyl), 7-(2,3-dihydro-benzo[b]furanyl)), benzo[b]thiophenyl (benzo[b]thiophen-2-yl, benzo[b]thiophen-3-yl, benzo[b]thiophen-4-yl, benzo[b]thiophen-5-yl, benzo[b]thiophen-6-yl, benzo[b]thiophen-7-yl), 2,3-dihydro-benzo[b]thiophenyl (2,3-dihydro-benzo[b]thiophen-2-yl, 2,3-dihydrobenzo[b]thiophen-3-yl, 2,3-dihydro-benzo[b]thiophen-4-yl, 2,3-dihydro-benzo[b]thiophen-5-yl, 2,3-dihydro-benzo[b]thiophen-6-yl, 2,3-dihydro-benzo[b]thiophen-7-yl), indolyl (1-indolyl, 2-indolyl, 3-indolyl, 4-indolyl, 5-indolyl, 6-indolyl, 7-indolyl), indazolyl (1-indazolyl, 3-indazolyl, 4-indazolyl, 5-indazolyl, 6-indazolyl, 7-indazolyl), benzimidazolyl (1-

benzimidazolyl, 2-benzimidazolyl, 4-benzimidazolyl, 5-benzimidazolyl, 6-benzimidazolyl, 7-benzimidazolyl, 8-benzimidazolyl), benzoxazolyl (2-benzoxazolyl, 3-benzoxazolyl, 4-benzoxazolyl, 5-benzoxazolyl, 6-benzoxazolyl, 7-benzoxazolyl), benzothiazolyl (2-benzothiazolyl, 4-benzothiazolyl, 5-benzothiazolyl, 6-benzothiazolyl, 7-benzothiazolyl), carbazolyl (1-carbazolyl, 2-carbazolyl, 3-carbazolyl, 4-carbazolyl), 5H-dibenz[b,f]azepinyl (5H-dibenz[b,f]azepin-1-yl, 5H-dibenz[b,f]azepine-2-yl, 5H-dibenz[b,f]azepine-3-yl, 5H-dibenz[b,f]azepine-4-yl, 5H-dibenz[b,f]azepine-5-yl), 10,11-dihydro-5H-dibenz[b,f]azepinyl (10,11-dihydro-5H-dibenz[b,f]azepine-1-yl, 10,11-dihydro-5H-dibenz[b,f]azepine-2-yl, 10,11-dihydro-5H-dibenz[b,f]azepine-3-yl, 10,11-dihydro-5H-dibenz[b,f]azepine-4-yl, 10,11-dihydro-5H-dibenz[b,f]azepine-5-yl), benzo[1,3]dioxole (2-benzo[1,3]dioxole, 4-benzo[1,3]dioxole, 5-benzo[1,3]dioxole, 6-benzo[1,3]dioxole, 7-benzo[1,3]dioxole), and tetrazolyl (5-tetrazolyl, N-tetrazolyl) as well as partly or fully saturated analogues of the ring systems mentioned above.

The term "fused aromatic ring system" as used herein, alone or in combination, refers to a carbocyclic aromatic ring radical fused to another carbocyclic aromatic ring radical, the two having two atoms in common. Typical fused aromatic ring systems include, but are not limited to naphthalene, quinoline, isoquinoline, indole, and isoindole.

A radical such as C_{x-y} -cycloalkyl- C_{a-b} -alkyl- shall designate that the radical's point of attachment is in part of the radical mentioned last.

As used herein, the term "optionally" means that the subsequently described event(s) may or may not occur, and includes both event(s) which occur and events that do not occur.

As used herein, the term "substituted" refers to substitution with the named substituent or substituents, multiple degrees of substitution being allowed unless otherwise stated.

Certain of the above defined terms may occur more than once in a given structural formulae, and upon such occurrence each term shall be defined independently of the other.

The term "nitro" shall mean the radical $-NO_2$.

The term "cyano" shall mean the radical $-CN$.

The term "halogen" shall mean $-Cl$, $-F$, $-Br$ or $-I$.

As used herein, the term "solvate" is a complex of variable stoichiometry formed by a solute (in this case, a compound according to the present invention) and a solvent. Such solvents for the purpose of the invention may not interfere with the biological activity of the solute. Solvents may be, by way of example, water, ethanol, or acetic acid.

As used herein, the term "prodrug" includes biohydrolyzable amides and biohydrolyzable esters and also encompasses a) compounds in which the biohydrolyzable functional-

ity in such a prodrug is encompassed in the compound according to the present invention, and b) compounds which may be oxidized or reduced biologically at a given functional group to yield drug substances according to the present invention. Examples of these functional groups include, but are not limited to, 1,4-dihydropyridine, N-alkylcarbonyl-1,4-

5 dihydropyridine, 1,4-cyclohexadiene, tert-butyl, and the like.

As used herein, the term "biohydrolyzable ester" is an ester of a drug substance (in this invention, a compound according to the invention) which either a) does not interfere with the biological activity of the parent substance but confers on that substance advantageous properties *in vivo* such as duration of action, onset of action, and the like, or b) is biologically
10 inactive but is readily converted *in vivo* by the subject to the biologically active principle. The advantage is that, for example, the biohydrolyzable ester is orally absorbed from the gut and is transformed to a compound according to the present invention in plasma. Many examples of such are known in the art and include by way of example lower alkyl esters (e.g., C₁-C₄), lower acyloxyalkyl esters, lower alkoxyacyloxyalkyl esters, alkoxyacyloxy esters, alkyl
15 acylamino alkyl esters, and choline esters.

As used herein, the term "biohydrolyzable amide" is an amide of a drug substance (in this invention, a compound according to the present invention) which either a) does not interfere with the biological activity of the parent substance but confers on that substance advantageous properties *in vivo* such as duration of action, onset of action, and the like, or b) is
20 biologically inactive but is readily converted *in vivo* by the subject to the biologically active principle. The advantage is that, for example, the biohydrolyzable amide is orally absorbed from the gut and is transformed to a compound according to the present invention in plasma. Many examples of such are known in the art and include by way of example lower alkyl amides, α -amino acid amides, alkoxyacyl amides, and alkylaminoalkylcarbonyl amides.

A "therapeutically effective amount" of a compound as used herein means an amount sufficient to cure, alleviate or partially arrest the clinical manifestations of a given disease and its complications. An amount adequate to accomplish this is defined as "therapeutically effective amount". Effective amounts for each purpose will depend on the severity of the disease or injury as well as the weight and general state of the subject. It will be un-
25 derstood that determining an appropriate dosage may be achieved using routine experimentation, by constructing a matrix of values and testing different points in the matrix.
30

The term "treatment" and "treating" as used herein means the management and care of a patient for the purpose of combating a condition, such as a disease or a disorder. The term is intended to include the full spectrum of treatments for a given condition from
35 which the patient is suffering, such as administration of the active compound to alleviate the

symptoms or complications, to delay the progression of the disease, disorder or condition, to alleviate or relief the symptoms and complications, and/or to cure or eliminate the disease, disorder or condition as well as to prevent the condition, wherein prevention is to be understood as the management and care of a patient for the purpose of combating the disease, condition, or disorder and includes the administration of the active compounds to prevent the onset of the symptoms or complications. The patient to be treated is preferably a mammal, in particular a human being.

DESCRIPTION OF THE INVENTION

The present invention provides the use of chemical uncouplers for enhancing mitochondrial respiration, which chemical uncouplers have an acceptably broad safety window.

More specifically, the present invention provides the use of a chemical compound with an E_{\max} in Assay (I), as described in the specification, of less than 75% of the E_{\max} of carbonylcyanide *p*-trifluoromethoxy-phenylhydrazone in Assay (I), or a pharmaceutically acceptable salt, solvate or prodrug thereof, for increasing mitochondrial respiration.

In a further embodiment, the present invention provides the use of a chemical compound with an E_{\max} in Assay (I), as described in the specification, of less than 70%, such as less than 65%, for instance less than 60%, such as less than 55%, for instance less than 50%, such as less than 45%, for instance less than 40%, such as less than 35%, for instance less than 30%, such as less than 25%, for instance less than 20%, such as less than 15%, for instance less than 10% of the E_{\max} of carbonylcyanide *p*-trifluoromethoxy-phenylhydrazone in Assay (I), or a pharmaceutically acceptable salt, solvate or prodrug thereof, for increasing mitochondrial respiration.

The present invention also provides the use of a chemical compound with an E_{\max} in Assay (I), as described in the specification, of less than 75% of the E_{\max} of 2,4-dinitrophenol (DNP) in Assay (I), or a pharmaceutically acceptable salt, solvate or prodrug thereof, for increasing mitochondrial respiration.

In a further embodiment, the present invention provides the use of a chemical compound with an E_{\max} in Assay (I), as described in the specification, of less than 70%, such as less than 65%, for instance less than 60%, such as less than 55%, for instance less than 50%, such as less than 45%, for instance less than 40%, such as less than 35%, for instance less than 30%, such as less than 25%, for instance less than 20%, such as less than 15%, for instance less than 10% of the E_{\max} of 2,4-dinitrophenol in Assay (I), or a pharmaceutically acceptable salt, solvate or prodrug thereof, for increasing mitochondrial respiration.

The E_{max} of the compounds, FCCP and DNP is calculated as described below under the heading "**Assay (I): Glucose utilisation in a human hepatocytes cell line (HEP-G2 cells)**".

5 The present invention also provides the use of a chemical compound with a slope calculated from the equation

$$X^n = (Y_2 - Y_0) / (Y_1 - Y_0)$$

wherein

Y_0 is the degree of stimulation measured as counts per minute (cpm) in Assay (I) in control samples without added test compound,

10 Y_1 is the degree of stimulation measured as cpm in Assay (I) with added test compound in a concentration of either $EC_{50}/2$ or $EC_{50}/3$,

Y_2 is the degree of stimulation measured as cpm in Assay (I) with added test compound in concentration of either $2xEC_{50}$ or $3xEC_{50}$,

X is 2 or 3 depending on which concentration of Y_1 and Y_2 used, and

15 n is the slope.

of a value equal to or less than the value for the slope calculated from the above equation with FCCP as test compound in Assay (I), or a pharmaceutically acceptable salt, solvate or prodrug thereof, for increasing mitochondrial respiration.

20 The equation should be understood such that when Y_1 is the degree of stimulation with added test compound in a concentration of $EC_{50}/2$, then Y_2 is the degree of stimulation with added test compound in concentration of $2xEC_{50}$, and X is 2, and when Y_1 is the degree of stimulation with added test compound in a concentration of $EC_{50}/3$, then Y_2 is the degree of stimulation with added test compound in a concentration of $3xEC_{50}$, and X is 3.

25 The values for use in the equation is calculated as described below under the heading: "**Assay (I): Glucose utilisation in a human hepatocytes cell line (HEP-G2 cells)**".

In a further embodiment, the slope is calculated by use of the computer software GraphPad Prism 3.0 (GraphPad software, Inc.).

In a further embodiment, the value for the slope calculated from the equation

$$X^n = (Y_2 - Y_0) / (Y_1 - Y_0)$$

30 is significantly less than the value for the slope calculated with FCCP as test compound in Assay (I).

35 The present invention also provides for the use of a compound with a Hill slope, n , calculated as describes in Assay IV herein which is lower than or equal to the Hill slope calculated for FCCP, or a pharmaceutically acceptable salt, solvate or prodrug thereof, for increasing mitochondrial respiration. In a further embodiment, said Hill slope for said com-

pound has a value of less than 95%, e.g. less than 90%, e.g. less than 80%, e.g. less than 70%, e.g. less than 50%, e.g. less than 40%, such as less than 20% of the value of said Hill slope calculated for FCCP.

The present invention also provides the use of a compound for which the value for the glucose utilisation caused by the compound in Assay (I) in concentrations of from CE_{max} to ten times CE_{max} does not fall significantly below the value of E_{max} for the compound in Assay (I), or a pharmaceutically acceptable salt, solvate or prodrug thereof, for increasing mitochondrial respiration. CE_{max} is calculated as described below under the heading: "**Assay (I): Glucose utilisation in a human hepatocytes cell line (HEP-G2 cells)**".

Increasing the mitochondrial respiration as described above may take place *in vitro* or *in vivo*, for instance in an assay or in a subject.

The present invention also provides the use of a chemical compound with an E_{max} in Assay (I), as described in the specification, of less than 75% of the E_{max} of carbonylcyanide *p*-trifluoromethoxy-phenylhydrazone in Assay (I), or a pharmaceutically acceptable salt, solvate or prodrug thereof, for treating a condition benefiting from an increase in mitochondrial respiration in a patient in need thereof.

In a further embodiment, the present invention provides the use of a chemical compound with an E_{max} in Assay (I), as described in the specification, of less than 70%, such as less than 65%, for instance less than 60%, such as less than 55%, for instance less than 50%, such as less than 45%, for instance less than 40% of the E_{max} of carbonylcyanide *p*-trifluoromethoxy-phenylhydrazone in Assay (I), or a pharmaceutically acceptable salt, solvate or prodrug thereof, for treating a condition benefiting from an increase in mitochondrial respiration in a patient in need thereof.

The present invention also provides the use of a chemical compound with an E_{max} in Assay (I), as described in the specification, of less than 75% of the E_{max} of 2,4-dinitrophenol in Assay (I), or a pharmaceutically acceptable salt, solvate or prodrug thereof, for treating a condition benefiting from an increase in mitochondrial respiration in a patient in need thereof.

In a further embodiment, the present invention provides the use of a chemical compound with an E_{max} in Assay (I), as described in the specification, of less than 70%, such as less than 65%, for instance less than 60%, such as less than 55%, for instance less than 50%, such as less than 45%, for instance less than 40% of the E_{max} of 2,4-dinitrophenol in Assay (I), or a pharmaceutically acceptable salt, solvate or prodrug thereof, for treating a condition benefiting from an increase in mitochondrial respiration in a patient in need thereof.

The E_{max} of the compounds, FCCP and DNP is calculated as described above.

The present invention also provides the use of a chemical compound with a slope calculated from the equation

$$X^n = (Y_2 - Y_0) / (Y_1 - Y_0)$$

wherein

5 Y_0 is the degree of stimulation measured as counts per minute (cpm) in Assay (I) in control samples without added test compound,

Y_1 is the degree of stimulation measured as cpm in Assay (I) with added test compound in a concentration of either $EC_{50}/2$ or $EC_{50}/3$,

10 Y_2 is the degree of stimulation measured as cpm in Assay (I) with added test compound in concentration of either $2xEC_{50}$ or $3xEC_{50}$,

X is 2 or 3 depending on which concentration of Y_1 and Y_2 used, and n is the slope.

of a value equal to or less than the value for the slope calculated from the above equation with FCCP as test compound in Assay (I), or a pharmaceutically acceptable salt, solvate or prodrug thereof, for treating a condition benefiting from an increase in mitochondrial respiration in a patient in need thereof.

The equation should be understood such that when Y_1 is the degree of stimulation with added test compound in a concentration of $EC_{50}/2$, then Y_2 is the degree of stimulation with added test compound in concentration of $2xEC_{50}$, and X is 2, and when Y_1 is the degree of stimulation with added test compound in a concentration of $EC_{50}/3$, then Y_2 is the degree of stimulation with added test compound in a concentration of $3xEC_{50}$, and X is 3.

The values for use in the equation is calculated as described below under the heading: "Assay (I): Glucose utilisation in a human hepatocytes cell line (HEP-G2 cells)".

In a further embodiment, the slope is calculated by use of the computer software 25 GraphPad Prism 3.0 (GraphPad software, Inc.).

In a further embodiment, the value for the slope calculated from the equation

$$X^n = (Y_2 - Y_0) / (Y_1 - Y_0)$$

is significantly less than the value for the slope calculated with FCCP as test compound in Assay (I).

30 The present invention also provides for the use of a compound with a Hill slope, n , calculated as describes in Assay IV herein which is lower than or equal to the Hill slope calculated for FCCP, or a pharmaceutically acceptable salt, solvate or prodrug thereof, for treating diseases benefiting from an increase in mitochondrial respiration. In a further embodiment, said Hill slope for said compound has a value of less than 95%, e.g. less than 90%,

e.g. less than 80%, e.g. less than 70%, e.g. less than 50%, e.g. less than 40%, such as less than 20% of the value of said Hill slope calculated for FCCP.

The present invention also provides the use of a compound for which the value for the glucose utilisation caused by the compound in Assay (I) in concentrations of from CE_{max} to ten times CE_{max} does not fall significantly below the value of E_{max} for the compound in Assay (I), or a pharmaceutically acceptable salt, solvate or prodrug thereof, for treating a condition benefiting from an increase in mitochondrial respiration in a patient in need thereof. CE_{max} is calculated as described below under the heading: "Assay (I): Glucose utilisation in a human hepatocytes cell line (HEP-G2 cells)".

The present invention also provides the use of a chemical compound with an E_{max} in Assay (I), as described in the specification, of less than 75% of the E_{max} of carbonylcyanide *p*-trifluoromethoxy-phenylhydrazide in Assay (I), or a pharmaceutically acceptable salt, solvate or prodrug thereof, for the preparation of a pharmaceutical composition for treating a condition benefiting from an increase in mitochondrial respiration in a patient in need thereof.

In a further embodiment, the present invention provides the use of a chemical compound with an E_{max} in Assay (I), as described in the specification, of less than 70%, such as less than 65%, for instance less than 60%, such as less than 55%, for instance less than 50%, such as less than 45%, for instance less than 40% of the E_{max} of carbonylcyanide *p*-trifluoromethoxy-phenylhydrazide in Assay (I), or a pharmaceutically acceptable salt, solvate or prodrug thereof, for the preparation of a pharmaceutical composition for treating a condition benefiting from an increase in mitochondrial respiration in a patient in need thereof.

The present invention also provides the use of a chemical compound with an E_{max} in Assay (I), as described in the specification, of less than 75% of the E_{max} of 2,4-dinitrophenol in Assay (I), or a pharmaceutically acceptable salt, solvate or prodrug thereof, for the preparation of a pharmaceutical composition for treating a condition benefiting from an increase in mitochondrial respiration in a patient in need thereof.

In a further embodiment, the present invention provides the use of a chemical compound with an E_{max} in Assay (I), as described in the specification, of less than 70%, such as less than 65%, for instance less than 60%, such as less than 55%, for instance less than 50%, such as less than 45%, for instance less than 40% of the E_{max} of 2,4-dinitrophenol in Assay (I), or a pharmaceutically acceptable salt, solvate or prodrug thereof, for the preparation of a pharmaceutical composition for treating a condition benefiting from an increase in mitochondrial respiration in a patient in need thereof.

The E_{max} of the compounds, FCCP and DNP is calculated as described above.

The present invention also provides the use of a chemical compound with a slope calculated from the equation

$$X^n = (Y_2 - Y_0) / (Y_1 - Y_0)$$

wherein

- 5 Y_0 is the degree of stimulation measured as counts per minute (cpm) in Assay (I) in control samples without added test compound,
 Y_1 is the degree of stimulation measured as cpm in Assay (I) with added test compound in a concentration of either $EC_{50}/2$ or $EC_{50}/3$,
 Y_2 is the degree of stimulation measured as cpm in Assay (I) with added test compound in concentration of either $2xEC_{50}$ or $3xEC_{50}$,
 10 X is 2 or 3 depending on which concentration of Y_1 and Y_2 used, and
 n is the slope.

- of a value equal to or less than the value for the slope calculated from the above equation with FCCP as test compound in Assay (I), or a pharmaceutically acceptable salt, solvate or
 15 prodrug thereof, for the preparation of a pharmaceutical composition for treating a condition benefiting from an increase in mitochondrial respiration in a patient in need thereof.

- The equation should be understood such that when Y_1 is the degree of stimulation with added test compound in a concentration of $EC_{50}/2$, then Y_2 is the degree of stimulation with added test compound in concentration of $2xEC_{50}$, and X is 2, and when Y_1 is the degree
 20 of stimulation with added test compound in a concentration of $EC_{50}/3$, then Y_2 is the degree of stimulation with added test compound in a concentration of $3xEC_{50}$, and X is 3.

The values for use in the equation is calculated as described below under the heading: "Assay (I): Glucose utilisation in a human hepatocytes cell line (HEP-G2 cells)".

- In a further embodiment, the slope is calculated by use of the computer software
 25 GraphPad Prism 3.0 (GraphPad software, Inc.).

In a further embodiment, the value for the slope calculated from the equation

$$X^n = (Y_2 - Y_0) / (Y_1 - Y_0)$$

is significantly less than the value for the slope calculated with FCCP as test compound in Assay (I).

- 30 The present invention also provides for the use of a compound with a Hill slope, n , calculated as describes in Assay IV herein which is lower than or equal to the Hill slope calculated for FCCP, or a pharmaceutically acceptable salt, solvate or prodrug thereof, for the preparation of a pharmaceutical composition for treating a condition benefiting from an increase in mitochondrial respiration. In a further embodiment, said Hill slope for said compound has a value of less than 95%, e.g. less than 90%, e.g. less than 80%, e.g. less than
 35

70%, e.g. less than 50%, e.g. less than 40%, such as less than 20% of the value of said Hill slope calculated for FCCP.

The present invention also provides the use of a compound for which the value for the glucose utilisation caused by the compound in Assay (I) in concentrations of from CE_{max} to ten times CE_{max} does not fall significantly below the value of E_{max} for the compound in Assay (I), or a pharmaceutically acceptable salt, solvate or prodrug thereof, for the preparation of a pharmaceutical composition for treating a condition benefiting from an increase in mitochondrial respiration in a patient in need thereof. CE_{max} is calculated as described below under the heading: "Assay (I): Glucose utilisation in a human hepatocytes cell line (HEP-G2 cells)".

The present invention also provides a method for treating a condition benefiting from an increase in mitochondrial respiration, which method comprises administering a therapeutically effective amount of a compound with an E_{max} in Assay (I), as described in the specification, of less than 75% of the E_{max} of carbonylcyanide *p*-trifluoromethoxy-phenylhydrazone in Assay (I), or a pharmaceutically acceptable salt, solvate or prodrug thereof, to a patient in need thereof.

In one embodiment, the present invention provides a method for treating a condition benefiting from an increase in mitochondrial respiration, which method comprises administering a therapeutically effective amount of a compound with an E_{max} in Assay (I), as described in the specification, of less than 70%, such as less than 65%, for instance less than 60%, such as less than 55%, for instance less than 50%, such as less than 45%, for instance less than 40%, such as less than 35%, for instance less than 30%, such as less than 25%, for instance less than 20%, such as less than 15%, for instance less than 10% of the E_{max} of carbonylcyanide *p*-trifluoromethoxy-phenylhydrazone in Assay (I), or a pharmaceutically acceptable salt, solvate or prodrug thereof, to a patient in need thereof.

In one embodiment, the present invention provides a method for treating a condition benefiting from an increase in mitochondrial respiration, which method comprises administering a therapeutically effective amount of a compound with an E_{max} in Assay (I), as described in the specification, of less than 75% of the E_{max} of 2,4-dinitrophenol (DNP) in Assay (I), or a pharmaceutically acceptable salt, solvate or prodrug thereof, to a patient in need thereof.

In one embodiment, the present invention provides a method for treating a condition benefiting from an increase in mitochondrial respiration, which method comprises administering a therapeutically effective amount of a compound with an E_{max} in Assay (I), as described in the specification, of less than 70%, such as less than 65%, for instance less than 60%, such as less than 55%, for instance less than 50%, such as less than 45%, for instance less

than 40%, such as less than 35%, for instance less than 30%, such as less than 25%, for instance less than 20%, such as less than 15%, for instance less than 10% of the E_{max} of 2,4-dinitrophenol (DNP) in Assay (I), or a pharmaceutically acceptable salt, solvate or prodrug thereof, to a patient in need thereof.

- 5 The E_{max} of the compounds, FCCP and DNP is calculated as described below under the heading **"Assay (I): Glucose utilisation in a human hepatocytes cell line (HEP-G2 cells)"**.

10 In one embodiment, the present invention provides a method for treating a condition benefiting from an increase in mitochondrial respiration, which method comprises administering a therapeutically effective amount of a compound with a slope calculated from the equation

$$X^n = (Y_2 - Y_0) / (Y_1 - Y_0)$$

wherein

15 Y_0 is the degree of stimulation measured as counts per minute (cpm) in Assay (I) in control samples without added test compound,

Y_1 is the degree of stimulation measured as cpm in Assay (I) with added test compound in a concentration of either $EC_{50}/2$ or $EC_{50}/3$,

Y_2 is the degree of stimulation measured as cpm in Assay (I) with added test compound in concentration of either $2 \times EC_{50}$ or $3 \times EC_{50}$,

20 X is 2 or 3 depending on which concentration of Y_1 and Y_2 used, and n is the slope.

of a value equal to or less than the value for the slope calculated from the above equation with FCCP as test compound in Assay (I), or a pharmaceutically acceptable salt, solvate or prodrug thereof, to a patient in need thereof.

25 The equation should be understood such that when Y_1 is the degree of stimulation with added test compound in a concentration of $EC_{50}/2$, then Y_2 is the degree of stimulation with added test compound in concentration of $2 \times EC_{50}$, and X is 2, and when Y_1 is the degree of stimulation with added test compound in a concentration of $EC_{50}/3$, then Y_2 is the degree of stimulation with added test compound in a concentration of $3 \times EC_{50}$, and X is 3.

30 The values for use in the equation is calculated as described below under the heading: **"Assay (I): Glucose utilisation in a human hepatocytes cell line (HEP-G2 cells)"**.

In a further embodiment, the slope is calculated by use of the computer software GraphPad Prism 3.0 (GraphPad software, Inc.).

In a further embodiment, the value for the slope calculated from the equation

35
$$X^n = (Y_2 - Y_0) / (Y_1 - Y_0)$$

is significantly less than the value for the slope calculated with FCCP as test compound in Assay (I).

The present invention also provides for a method for treating a condition benefiting from an increase in mitochondrial respiration, which method comprises administering a therapeutically effective amount of a compound with a Hill slope, n , calculated as describes in Assay IV herein which is lower than or equal to the Hill slope calculated for FCCP, or a pharmaceutically acceptable salt, solvate or prodrug thereof, to a patient in need thereof. In a further embodiment, said Hill slope for said compound has a value of less than 95%, e.g. less than 90%, e.g. less than 80%, e.g. less than 70%, e.g. less than 50%, e.g. less than 40%, such as less than 20% of the value of said Hill slope calculated for FCCP.

In one embodiment, the present invention provides a method for treating a condition benefiting from an increase in mitochondrial respiration, which method comprises administering a therapeutically effective amount of a compound for which the value for the glucose utilisation caused by the compound in Assay (I) in concentrations of from CE_{max} to ten times CE_{max} does not fall significantly below the value of E_{max} for the compound in Assay (I), or a pharmaceutically acceptable salt, solvate or prodrug thereof, to a patient in need thereof. CE_{max} is calculated as described below under the heading: **"Assay (I): Glucose utilisation in a human hepatocytes cell line (HEP-G2 cells)"**.

Such conditions as referred to above as being conditions benefiting from an increase in mitochondrial respiration, may be such conditions as obesity, atherosclerosis, hypertension, diabetes, coronary heart disease, gallbladder disease, osteoarthritis, and cancers such as endometrial, breast, prostate and colon cancers, especially, obesity, type 2 diabetes (especially in obese patients), and dyslipidemia (especially in obese patients).

The subject may be any mammal suffering from a condition benefiting from increased mitochondrial respiration. Such mammals may include, for instance, horses, cows, sheep, pigs, mice, dogs, cats, primates such as chimpanzees, gorillas, rhesus monkeys, and, most preferably, humans.

Use of the compounds according to the present invention in the treatment of obesity may very likely reduce or eliminate the side effects such as irritation of the skin, glaucoma etc. known from treatment of obesity with DNP and other chemical uncouplers with narrow safety windows.

The present invention also provides the use of a chemical compound with an E_{max} in Assay (I), as described in the specification, of less than 75% of the E_{max} of carbonylcyanide *p*-trifluoromethoxy-phenylhydrazone in Assay (I), or a pharmaceutically acceptable salt, solvate or prodrug thereof, for reduction of reactive oxygen species.

In a further embodiment, the present invention provides the use of a chemical compound with an E_{\max} in Assay (I), as described in the specification, of less than 70%, such as less than 65%, for instance less than 60%, such as less than 55%, for instance less than 50%, such as less than 45%, for instance less than 40%, such as less than 35%, for instance less than 30%, such as less than 25%, for instance less than 20%, such as less than 15%, for instance less than 10% of the E_{\max} of carbonylcyanide *p*-trifluoromethoxy-phenylhydrazone in Assay (I), or a pharmaceutically acceptable salt, solvate or prodrug thereof, for reduction of reactive oxygen species.

The present invention also provides the use of a chemical compound with an E_{\max} in Assay (I), as described in the specification, of less than 75% of the E_{\max} of 2,4-dinitrophenol (DNP) in Assay (I), or a pharmaceutically acceptable salt, solvate or prodrug thereof, for reduction of reactive oxygen species.

In a further embodiment, the present invention provides the use of a chemical compound with an E_{\max} in Assay (I), as described in the specification, of less than 70%, such as less than 65%, for instance less than 60%, such as less than 55%, for instance less than 50%, such as less than 45%, for instance less than 40%, such as less than 35%, for instance less than 30%, such as less than 25%, for instance less than 20%, such as less than 15%, for instance less than 10% of the E_{\max} of 2,4-dinitrophenol in Assay (I), or a pharmaceutically acceptable salt, solvate or prodrug thereof, for reduction of reactive oxygen species.

The E_{\max} of the compounds, FCCP and DNP is calculated as described below under the heading "**Assay (I): Glucose utilisation in a human hepatocytes cell line (HEP-G2 cells)**".

The present invention also provides the use of a chemical compound with a slope calculated from the equation

$$X^n = (Y_2 - Y_0) / (Y_1 - Y_0)$$

wherein

Y_0 is the degree of stimulation measured as counts per minute (cpm) in Assay (I) in control samples without added test compound,

Y_1 is the degree of stimulation measured as cpm in Assay (I) with added test compound in a concentration of either $EC_{50}/2$ or $EC_{50}/3$,

Y_2 is the degree of stimulation measured as cpm in Assay (I) with added test compound in concentration of either $2 \times EC_{50}$ or $3 \times EC_{50}$.

X is 2 or 3 depending on which concentration of Y_1 and Y_2 used, and

n is the slope.

of a value equal to or less than the value for the slope calculated from the above equation with FCCP as test compound in Assay (I), or a pharmaceutically acceptable salt, solvate or prodrug thereof, for reduction of reactive oxygen species.

5 The equation should be understood such that when Y_1 is the degree of stimulation with added test compound in a concentration of $EC_{50}/2$, then Y_2 is the degree of stimulation with added test compound in concentration of $2xEC_{50}$, and X is 2, and when Y_1 is the degree of stimulation with added test compound in a concentration of $EC_{50}/3$, then Y_2 is the degree of stimulation with added test compound in a concentration of $3xEC_{50}$, and X is 3.

10 The values for use in the equation is calculated as described below under the heading: **"Assay (I): Glucose utilisation in a human hepatocytes cell line (HEP-G2 cells)"**.

In a further embodiment, the slope is calculated by use of the computer software GraphPad Prism 3.0 (GraphPad software, Inc.).

In a further embodiment, the value for the slope calculated from the equation

$$X^n = (Y_2 - Y_0) / (Y_1 - Y_0)$$

15 is significantly less than the value for the slope calculated with FCCP as test compound in Assay (I).

20 The present invention also provides for the use of a compound with a Hill slope, n , calculated as describes in Assay IV herein which is lower than or equal to the Hill slope calculated for FCCP, or a pharmaceutically acceptable salt, solvate or prodrug thereof, for reduction of reactive oxygen species. In a further embodiment, said Hill slope for said compound has a value of less than 95%, e.g. less than 90%, e.g. less than 80%, e.g. less than 70%, e.g. less than 50%, e.g. less than 40%, such as less than 20% of the value of said Hill slope calculated for FCCP.

25 The present invention also provides the use of a compound for which the value for the glucose utilisation caused by the compound in Assay (I) in concentrations of from CE_{max} to ten times CE_{max} does not fall significantly below the value of E_{max} for the compound in Assay (I), or a pharmaceutically acceptable salt, solvate or prodrug thereof, for reduction of reactive oxygen species. CE_{max} is calculated as described below under the heading: **"Assay (I): Glucose utilisation in a human hepatocytes cell line (HEP-G2 cells)"**.

30 Reduction of reactive oxygen species as described above may take place *in vitro* or *in vivo*, for instance in an assay or in a subject.

The present invention also provides the use of a chemical compound with an E_{max} in Assay (I), as described in the specification, of less than 75% of the E_{max} of carbonylcyanide *p*-trifluoromethoxy-phenylhydrazone in Assay (I), or a pharmaceutically acceptable salt, sol-

vate or prodrug thereof, for treating a condition benefiting from a reduction of reactive oxygen species in a patient in need thereof.

In a further embodiment, the present invention provides the use of a chemical compound with an E_{\max} in Assay (I), as described in the specification, of less than 70%, such as less than 65%, for instance less than 60%, such as less than 55%, for instance less than 50%, such as less than 45%, for instance less than 40%, such as less than 35%, for instance less than 30%, such as less than 25%, for instance less than 20%, such as less than 15%, for instance less than 10% of the E_{\max} of carbonylcyanide *p*-trifluoromethoxyphenylhydrazone in Assay (I), or a pharmaceutically acceptable salt, solvate or prodrug thereof, for treating a condition benefiting from a reduction of reactive oxygen species in a patient in need thereof.

The present invention also provides the use of a chemical compound with an E_{\max} in Assay (I), as described in the specification, of less than 75% of the E_{\max} of 2,4-dinitrophenol (DNP) in Assay (I), or a pharmaceutically acceptable salt, solvate or prodrug thereof, for treating a condition benefiting from a reduction of reactive oxygen species in a patient in need thereof.

In a further embodiment, the present invention provides the use of a chemical compound with an E_{\max} in Assay (I), as described in the specification, of less than 70%, such as less than 65%, for instance less than 60%, such as less than 55%, for instance less than 50%, such as less than 45%, for instance less than 40%, such as less than 35%, for instance less than 30%, such as less than 25%, for instance less than 20%, such as less than 15%, for instance less than 10% of the E_{\max} of 2,4-dinitrophenol in Assay (I), or a pharmaceutically acceptable salt, solvate or prodrug thereof, for treating a condition benefiting from a reduction of reactive oxygen species in a patient in need thereof.

The E_{\max} of the compounds, FCCP and DNP is calculated as described above.

The present invention also provides the use of a chemical compound with a slope calculated from the equation

$$X^n = (Y_2 - Y_0) / (Y_1 - Y_0)$$

wherein

Y_0 is the degree of stimulation measured as counts per minute (cpm) in Assay (I) in control samples without added test compound,

Y_1 is the degree of stimulation measured as cpm in Assay (I) with added test compound in a concentration of either $EC_{50}/2$ or $EC_{50}/3$,

Y_2 is the degree of stimulation measured as cpm in Assay (I) with added test compound in concentration of either $2 \times EC_{50}$ or $3 \times EC_{50}$.

X is 2 or 3 depending on which concentration of Y_1 and Y_2 used, and n is the slope.

of a value equal to or less than the value for the slope calculated from the above equation with FCCP as test compound in Assay (I), or a pharmaceutically acceptable salt, solvate or prodrug thereof, for treating a condition benefiting from a reduction of reactive oxygen species in a patient in need thereof.

The equation should be understood such that when Y_1 is the degree of stimulation with added test compound in a concentration of $EC_{50}/2$, then Y_2 is the degree of stimulation with added test compound in concentration of $2xEC_{50}$, and X is 2, and when Y_1 is the degree of stimulation with added test compound in a concentration of $EC_{50}/3$, then Y_2 is the degree of stimulation with added test compound in a concentration of $3xEC_{50}$, and X is 3.

The values for use in the equation is calculated as described below under the heading: **"Assay (I): Glucose utilisation in a human hepatocytes cell line (HEP-G2 cells)"**.

In a further embodiment, the slope is calculated by use of the computer software GraphPad Prism 3.0 (GraphPad software, Inc.).

In a further embodiment, the value for the slope calculated from the equation

$$X^n = (Y_2 - Y_0) / (Y_1 - Y_0)$$

is significantly less than the value for the slope calculated with FCCP as test compound in Assay (I).

The present invention also provides for the use of a compound with a Hill slope, n, calculated as describes in Assay IV herein which is lower than or equal to the Hill slope calculated for FCCP, or a pharmaceutically acceptable salt, solvate or prodrug thereof, for treating a disease benefiting from a reduction in reactive oxygen. In a further embodiment, said Hill slope for said compound has a value of less than 95%, e.g. less than 90%, e.g. less than 80%, e.g. less than 70%, e.g. less than 50%, e.g. less than 40%, such as less than 20% of the value of said Hill slope calculated for FCCP.

The present invention also provides the use of a compound for which the value for the glucose utilisation caused by the compound in Assay (I) in concentrations of from CE_{max} to ten times CE_{max} does not fall significantly below the value of E_{max} for the compound in Assay (I), or a pharmaceutically acceptable salt, solvate or prodrug thereof, for treating a condition benefiting from a reduction of reactive oxygen species in a patient in need thereof. CE_{max} is calculated as described below under the heading: **"Assay (I): Glucose utilisation in a human hepatocytes cell line (HEP-G2 cells)"**.

The present invention also provides the use of a chemical compound with an E_{max} in Assay (I), as described in the specification, of less than 75% of the E_{max} of carbonylcyanide

p-trifluoromethoxy-phenylhydrazone in Assay (I), or a pharmaceutically acceptable salt, solvate or prodrug thereof, for the preparation of a pharmaceutical composition for treating a condition benefiting from a reduction of reactive oxygen species in a patient in need thereof.

In a further embodiment, the present invention provides the use of a chemical compound with an E_{max} in Assay (I), as described in the specification, of less than 70%, such as less than 65%, for instance less than 60%, such as less than 55%, for instance less than 50%, such as less than 45%, for instance less than 40%, such as less than 35%, for instance less than 30%, such as less than 25%, for instance less than 20%, such as less than 15%, for instance less than 10% of the E_{max} of carbonylcyanide *p*-trifluoromethoxy-phenylhydrazone in Assay (I), or a pharmaceutically acceptable salt, solvate or prodrug thereof, for the preparation of a pharmaceutical composition for treating a condition benefiting from a reduction of reactive oxygen species in a patient in need thereof.

The present invention also provides the use of a chemical compound with an E_{max} in Assay (I), as described in the specification, of less than 75% of the E_{max} of 2,4-dinitrophenol (DNP) in Assay (I), or a pharmaceutically acceptable salt, solvate or prodrug thereof, for the preparation of a pharmaceutical composition for treating a condition benefiting from a reduction of reactive oxygen species in a patient in need thereof.

In a further embodiment, the present invention provides the use of a chemical compound with an E_{max} in Assay (I), as described in the specification, of less than 70%, such as less than 65%, for instance less than 60%, such as less than 55%, for instance less than 50%, such as less than 45%, for instance less than 40%, such as less than 35%, for instance less than 30%, such as less than 25%, for instance less than 20%, such as less than 15%, for instance less than 10% of the E_{max} of 2,4-dinitrophenol in Assay (I), or a pharmaceutically acceptable salt, solvate or prodrug thereof, for the preparation of a pharmaceutical composition for treating a condition benefiting from a reduction of reactive oxygen species in a patient in need thereof.

The E_{max} of the compounds, FCCP and DNP is calculated as described above.

The present invention also provides the use of a chemical compound with a slope calculated from the equation

$$X^n = (Y_x - Y_0) / (Y_1 - Y_0)$$

wherein

Y_0 is the degree of stimulation measured as counts per minute (cpm) in Assay (I) in control samples without added test compound,

Y_1 is the degree of stimulation measured as cpm in Assay (I) with added test compound in a concentration of either $EC_{50}/2$ or $EC_{50}/3$,

Y_2 is the degree of stimulation measured as cpm in Assay (I) with added test compound in concentration of either $2 \times EC_{50}$ or $3 \times EC_{50}$,

X is 2 or 3 depending on which concentration of Y_1 and Y_2 used, and

n is the slope.

- 5 of a value equal to or less than the value for the slope calculated from the above equation with FCCP as test compound in Assay (I), or a pharmaceutically acceptable salt, solvate or prodrug thereof, for the preparation of a pharmaceutical composition for treating a condition benefiting from a reduction of reactive oxygen species in a patient in need thereof.

- 10 The equation should be understood such that when Y_1 is the degree of stimulation with added test compound in a concentration of $EC_{50}/2$, then Y_2 is the degree of stimulation with added test compound in concentration of $2 \times EC_{50}$, and X is 2, and when Y_1 is the degree of stimulation with added test compound in a concentration of $EC_{50}/3$, then Y_2 is the degree of stimulation with added test compound in a concentration of $3 \times EC_{50}$, and X is 3.

- 15 The values for use in the equation is calculated as described below under the heading: "Assay (I): Glucose utilisation in a human hepatocytes cell line (HEP-G2 cells)".

In a further embodiment, the slope is calculated by use of the computer software GraphPad Prism 3.0 (GraphPad software, Inc.).

In a further embodiment, the value for the slope calculated from the equation

$$X^n = (Y_2 - Y_0) / (Y_1 - Y_0)$$

- 20 is significantly less than the value for the slope calculated with FCCP as test compound in Assay (I).

- 25 The present invention also provides for the use of a compound with a Hill slope, n, calculated as describes in Assay IV herein which is lower than or equal to the Hill slope calculated for FCCP, or a pharmaceutically acceptable salt, solvate or prodrug thereof, for the preparation of a pharmaceutical composition for treating a condition benefiting from a reduction in reactive oxygen species. In a further embodiment, said Hill slope for said compound has a value of less than 95%, e.g. less than 90%, e.g. less than 80%, e.g. less than 70%, e.g. less than 50%, e.g. less than 40%, such as less than 20% of the value of said Hill slope calculated for FCCP.

- 30 The present invention also provides the use of a compound for which the value for the glucose utilisation caused by the compound in Assay (I) in concentrations of from CE_{max} to ten times CE_{max} does not fall significantly below the value of E_{max} for the compound in Assay (I), or a pharmaceutically acceptable salt, solvate or prodrug thereof, for the preparation of a pharmaceutical composition for treating a condition benefiting from a reduction of reactive oxygen species in a patient in need thereof. CE_{max} is calculated as described below un-
- 35

der the heading: "Assay (I): Glucose utilisation in a human hepatocytes cell line (HEP-G2 cells)".

The present invention also provides a method for treating a condition benefiting from a reduction of reactive oxygen species, which method comprises administering a therapeutically effective amount of a compound with an E_{\max} in Assay (I), as described in the specification, of less than 75% of the E_{\max} of carbonylcyanide *p*-trifluoromethoxy-phenylhydrazone in Assay (I), or a pharmaceutically acceptable salt, solvate or prodrug thereof, to a patient in need thereof.

In one embodiment, the present invention provides a method for treating a condition benefiting from a reduction of reactive oxygen species, which method comprises administering a therapeutically effective amount of a compound with an E_{\max} in Assay (I), as described in the specification, of less than 70%, such as less than 65%, for instance less than 60%, such as less than 55%, for instance less than 50%, such as less than 45%, for instance less than 40%, such as less than 35%, for instance less than 30%, such as less than 25%, for instance less than 20%, such as less than 15%, for instance less than 10% of the E_{\max} of carbonylcyanide *p*-trifluoromethoxy-phenylhydrazone in Assay (I), or a pharmaceutically acceptable salt, solvate or prodrug thereof, to a patient in need thereof.

In one embodiment, the present invention provides a method for treating a condition benefiting from a reduction of reactive oxygen species, which method comprises administering a therapeutically effective amount of a compound with an E_{\max} in Assay (I), as described in the specification, of less than 75% of the E_{\max} of 2,4-dinitrophenol (DNP) in Assay (I), or a pharmaceutically acceptable salt, solvate or prodrug thereof, to a patient in need thereof.

In one embodiment, the present invention provides a method for treating a condition benefiting from a reduction of reactive oxygen species, which method comprises administering a therapeutically effective amount of a compound with an E_{\max} in Assay (I), as described in the specification, of less than 70%, such as less than 65%, for instance less than 60%, such as less than 55%, for instance less than 50%, such as less than 45%, for instance less than 40%, such as less than 35%, for instance less than 30%, such as less than 25%, for instance less than 20%, such as less than 15%, for instance less than 10% of the E_{\max} of 2,4-dinitrophenol (DNP) in Assay (I), or a pharmaceutically acceptable salt, solvate or prodrug thereof, to a patient in need thereof.

The E_{\max} of the compounds, FCCP and DNP is calculated as described below under the heading "Assay (I): Glucose utilisation in a human hepatocytes cell line (HEP-G2 cells)".

In one embodiment, the present invention provides a method for treating a condition benefiting from a reduction of reactive oxygen species, which method comprises administering a therapeutically effective amount of a compound with a slope calculated from the equation

$$X^n = (Y_2 - Y_0) / (Y_1 - Y_0)$$

wherein

Y_0 is the degree of stimulation measured as counts per minute (cpm) in Assay (I) in control samples without added test compound,

Y_1 is the degree of stimulation measured as cpm in Assay (I) with added test compound in a concentration of either $EC_{50}/2$ or $EC_{50}/3$,

Y_2 is the degree of stimulation measured as cpm in Assay (I) with added test compound in concentration of either $2xEC_{50}$ or $3xEC_{50}$,

X is 2 or 3 depending on which concentration of Y_1 and Y_2 used, and

n is the slope.

of a value equal to or less than the value for the slope calculated from the above equation with FCCP as test compound in Assay (I), or a pharmaceutically acceptable salt, solvate or prodrug thereof, to a patient in need thereof.

The equation should be understood such that when Y_1 is the degree of stimulation with added test compound in a concentration of $EC_{50}/2$, then Y_2 is the degree of stimulation with added test compound in concentration of $2xEC_{50}$, and X is 2, and when Y_1 is the degree of stimulation with added test compound in a concentration of $EC_{50}/3$, then Y_2 is the degree of stimulation with added test compound in a concentration of $3xEC_{50}$, and X is 3.

The values for use in the equation is calculated as described below under the heading: "**Assay (I): Glucose utilisation in a human hepatocytes cell line (HEP-G2 cells)**".

In a further embodiment, the slope is calculated by use of the computer software GraphPad Prism 3.0 (GraphPad software, Inc.).

In a further embodiment, the value for the slope calculated from the equation

$$X^n = (Y_2 - Y_0) / (Y_1 - Y_0)$$

is significantly less than the value for the slope calculated with FCCP as test compound in Assay (I).

The present invention also provides for the a method of treating a condition benefiting from a reduction in reactive oxygen species, which method comprises administering a therapeutically effective amount of a compound with a Hill slope, n , calculated as describes in Assay IV herein which is lower than or equal to the Hill slope calculated for FCCP, or a pharmaceutically acceptable salt, solvate or prodrug thereof, to a patient in need thereof. In a

further embodiment, said Hill slope for said compound has a value of less than 95%, e.g. less than 90%, e.g. less than 80%, e.g. less than 70%, e.g. less than 50%, e.g. less than 40%, such as less than 20% of the value of said Hill slope calculated for FCCP.

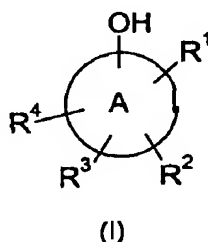
In one embodiment, the present invention provides a method for treating a condition benefiting from a reduction of reactive oxygen species, which method comprises administering a therapeutically effective amount of a compound for which the value for the glucose utilisation caused by the compound in Assay (I) in concentrations of from CE_{max} to ten times CE_{max} does not fall significantly below the value of E_{max} for the compound in Assay (I), or a pharmaceutically acceptable salt, solvate or prodrug thereof, to a patient in need thereof.

CE_{max} is calculated as described below under the heading: "Assay (I): Glucose utilisation in a human hepatocytes cell line (HEP-G2 cells)".

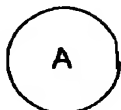
Such conditions as referred to above as being conditions benefiting from a reduction of reactive oxygen species may be such conditions as the aging process, damage of heart tissue as well as damage of neuronal tissue.

The subject may be any mammal suffering from a condition benefiting from a reduction of reactive oxygen species. Such mammals may include, for instance, horses, cows, sheep, pigs, mice, dogs, cats, primates such as chimpanzees, gorillas, rhesus monkeys, and, most preferably, humans.

In one embodiment of the present invention, the compound is of the general formula (I)



wherein



is an aryl, or heteroaryl,

R^1 is halogen, $-CHO$, $-CO_2R^{32}$, $-COR^{32}$, $-SO_3H$, $-CCl_3$, $-CF_3$, $-NO$, $-NO_2$, $-CN$, $-CH=CH-R^{33}$, $-C(R^{33})(R^{34})$, $-SOR^{32}$, $-SO_2R^{32}$ or aryl substituted with from one to five substituents selected from halogen, $-CHO$, $-CO_2R^{32}$, $-COR^{32}$, $-SO_3H$, $-CCl_3$, $-CF_3$, $-NO$, $-NO_2$, $-CN$, $-CH=CH-R^{33}$, $-CH(R^{33})(R^{34})$, $-SOR^{32}$, $-SO_2R^{32}$, wherein

R^{32} is hydrogen, alkyl, aryl, or heteroaryl; and

R^{33} and R^{34} independently of each other are halogen, $-\text{CHO}$, $-\text{CO}_2R^{35}$, $-\text{COR}^{35}$, $-\text{SO}_3\text{H}$, $-\text{CCl}_3$, $-\text{CF}_3$, $-\text{NO}$, $-\text{NO}_2$, $-\text{CN}$, $-\text{SOR}^{35}$, $-\text{SO}_2R^{35}$, wherein

R^{35} is hydrogen or alkyl;

- 5 and is attached on a carbon atom adjacent to the carbon atom to which the hydroxy group is attached;

R^2 is $\text{C}(\text{X})_3$, NO_2 , alkyl, nitro, halogen, alkyl-O-, alkyl-C(O)-, alkyl-C(O)-O-, or aryl, wherein

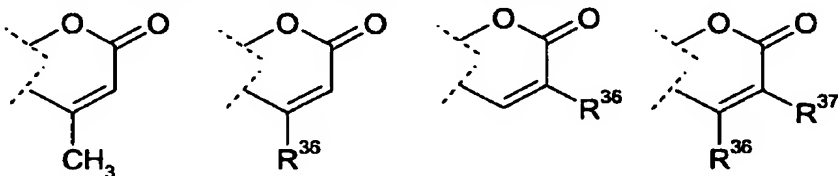
X is halogen; and

R^3 and R^4 independently of each other are hydrogen, alkyl, nitro, halogen, alkyl-O-, alkyl-

- 10 C(O)-, alkyl-C(O)-O-, or aryl;

or

R^2 and R^3 together forms one of the the diradicals



wherein

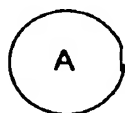
- 15 R^{36} and R^{37} , independently of each other, are hydrogen, halogen, $\text{C}(\text{X})_3$, nitro, cyano, alkyl, alkyl-O-, alkyl-C(O)-, or aryl, wherein

X is halogen;

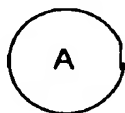
and where the two connecting atoms are connected to adjacent carbon atoms; and

R^4 is hydrogen, halogen, $\text{C}(\text{X})_3$, nitro, cyano, alkyl, alkyl-O-, alkyl-C(O)-, or aryl.

- 20 In cases where



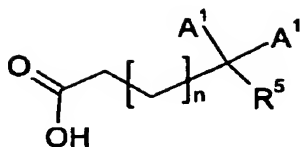
is a heteroaryl, the substituents R^1 , R^2 , R^3 and R^4 are preferably not attached to any of the heteroatoms in



- 25 Further embodiments of this embodiment are clear from the appended claims.

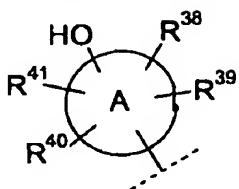
In one embodiment of the present invention, the compound is of the general formula (II)

28

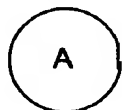


(II)

wherein A¹ is



, wherein



5

is an aryl, or heteroaryl,

R³⁸ is halogen, -CHO, -CO₂R⁴², -COR⁴², -SO₃H, -CCl₃, -CF₃, -NO, -NO₂, -CN, -SOR⁴², or -SO₂R⁴², wherein

R⁴² is hydrogen or alkyl;

10

and is attached to a carbon atom adjacent to the carbon atom to which the hydroxy group is attached;

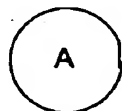
R³⁹, R⁴⁰, and R⁴¹ independently of each other are hydrogen, alkyl, nitro, cyano, halogen, alkyl-O-, alkyl-C(O)-, alkyl-C(O)-O-, or aryl;

R⁵ is hydrogen or alkyl; and

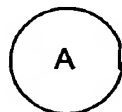
15

n is an integer of from 0 to 10.

In cases where



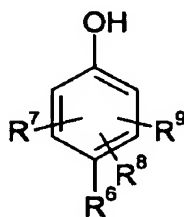
is a heteroaryl, the substituents R³⁸, R³⁹, R⁴⁰, and R⁴¹ are preferably not attached to any of the heteroatoms in



20

Further embodiments of this embodiment are clear from the appended claims.

In one embodiment of the present invention, the compound is of the general formula (III)



(III)

5 wherein

R^6 is halogen, $-\text{CHO}$, $-\text{CO}_2R^{43}$, $-\text{COR}^{43}$, $-\text{SO}_3\text{H}$, $-\text{CCl}_3$, $-\text{CF}_3$, $-\text{NO}$, $-\text{NO}_2$, $-\text{CN}$, $-\text{CH}=\text{CH}-R^{44}$, $-\text{C}(R^{44})(R^{45})$, $-\text{SOR}^{43}$, $-\text{SO}_2R^{43}$ or aryl substituted with from one to five substituents selected from halogen, $-\text{CHO}$, $-\text{CO}_2R^{43}$, $-\text{COR}^{43}$, $-\text{SO}_3\text{H}$, $-\text{CCl}_3$, $-\text{CF}_3$, $-\text{NO}$, $-\text{NO}_2$, $-\text{CN}$, $-\text{CH}=\text{CH}-R^{44}$, $-\text{CH}(R^{44})(R^{45})$, $-\text{SOR}^{43}$, $-\text{SO}_2R^{43}$, wherein

10 R^{43} is hydrogen or alkyl; and

R^{44} and R^{45} independently of each other are halogen, $-\text{CHO}$, $-\text{CO}_2R^{46}$, $-\text{COR}^{46}$, $-\text{SO}_3\text{H}$, $-\text{CCl}_3$, $-\text{CF}_3$, $-\text{NO}$, $-\text{NO}_2$, $-\text{CN}$, $-\text{SOR}^{46}$, $-\text{SO}_2R^{46}$, wherein

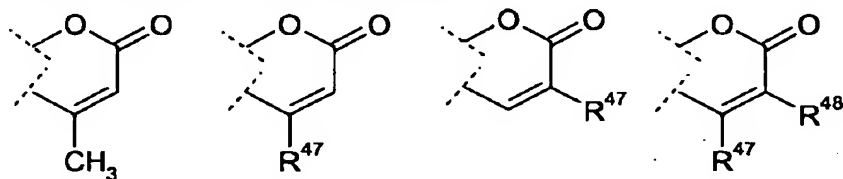
R^{46} is hydrogen, alkyl, or aryl;

R^7 is alkyl, nitro, halogen, alkyl-O-, alkyl-C(O)-, or alkyl-C(O)-O-; and

15 R^8 and R^9 independently of each other are hydrogen, alkyl, nitro, halogen, alkyl-O-, alkyl-C(O)-, alkyl-C(O)-O-, or aryl;

or

R^7 and R^8 together forms the diradical



20 wherein R^{47} and R^{48} , independently of each other, are hydrogen, alkyl, nitro, halogen, alkyl-O-, alkyl-C(O)-, or alkyl-C(O)-O-,

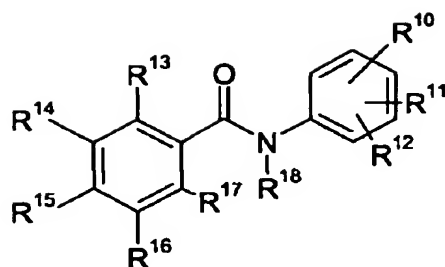
where the two valence atoms are connected to adjacent carbon atoms; and

R^9 is hydrogen, alkyl, nitro, halogen, alkyl-O-, or alkyl-C(O)-.

Further embodiments of this embodiment are clear from the appended claims.

25 In one embodiment of the present invention, the compound is of the general formula (IV)

30



(IV)

wherein

R¹⁰, R¹¹ and R¹² independently of each other are hydrogen, trifluoromethyl, nitro, cyano, alkyl-S-, SO_y, R⁴⁹-O-, N(R⁵⁰)(R⁵¹)-, alkyl, halogen, or aryl-S-, wherein y is an integer of 1 or 2;

R⁴⁹, R⁵⁰ and R⁵¹ independently of each other are hydrogen or alkyl;

wherein at least one of R¹⁰, R¹¹ and R¹² is different from hydrogen;

and

R¹³, R¹⁴, R¹⁵, R¹⁶ and R¹⁷ independently of each other are hydrogen, halogen, hydroxy, halogen, cyano, or alkyl, aryl, aryl-S-, or heteroaryl, optionally substituted with halogen;

or

R¹³ and R¹⁴ together form a conjugated alkenylene, which together with the benzene ring forms a fused aromatic ring system, which may optionally be substituted with one or more substituents selected from the group consisting of hydroxy, methyl, halogen, CF₃, alkyl-O-, nitro, and cyano;

and

R¹⁵, R¹⁶ and R¹⁷, independently of each other, are hydrogen, halogen, hydroxy, halogen, or alkyl optionally substituted with halogen

or

R¹⁴ and R¹⁵ together form a conjugated alkenylene, which together with the benzene ring forms a fused aromatic ring system, which may optionally be substituted with one or more substituents selected from the group consisting of hydroxy, methyl, halogen, CF₃, alkyl-O-, nitro, and cyano;

and

R¹³, R¹⁶ and R¹⁷ independently of each other are hydrogen, halogen, hydroxy, halogen, or alkyl, aryl or heteroaryl, optionally substituted with halogen;

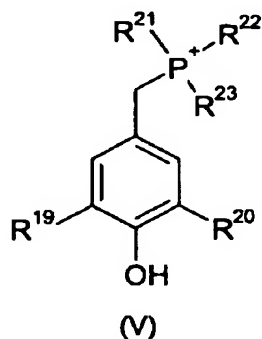
and

R^{18} is hydrogen.

Further embodiments of this embodiment are clear from the appended claims.

In one embodiment of the present invention, the compound is of the general for-

5 mula (V)



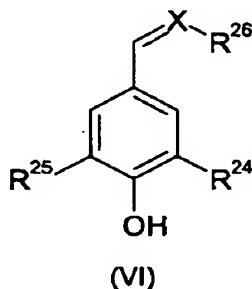
wherein R^{19} and R^{20} independently of each other are alkyl;

and

10 R^{21} , R^{22} and R^{23} independently of each other are selected from alkyl, cycloalkyl, or aryl.

Further embodiments of this embodiment are clear from the appended claims.

In one embodiment of the present invention, the compound is of the general formula (VI)



wherein

R^{24} and R^{25} independently of each other are alkyl or cycloalkyl;

and

X is $=C(R^{52})-$; wherein

20 R^{52} is hydrogen, cyano, nitro, $R^{53}-S(O)_2-$, tetrazole, alkyl-S-, alkyl-C(O)-, or alkyl-O-C(O)-, wherein

R^{53} is hydrogen, or alkyl or phenyl optionally substituted with halogen; and

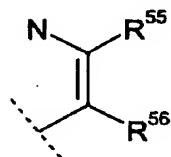
R^{26} is cyano, nitro, $R^{54}-S(O)_2-$, tetrazole, alkyl-C(O)-, or alkyl-O-C(O)-, wherein

R^{54} is hydrogen, or alkyl or phenyl optionally substituted with halogen;

or

X is =N-, and

R^{26} is cyano, nitro, R^{54} -S(O)₂-, alkyl-C(O)-, alkyl-O-C(O)-, or



wherein

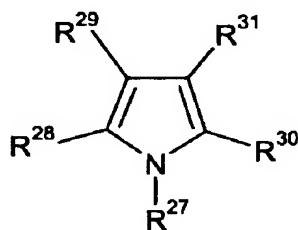
R^{54} is hydrogen, or alkyl or phenyl optionally substituted with halogen; and

R^{55} and R^{56} independently of each other are cyano, nitro, R^{57} -S(O)₂-, alkyl-C(O)-, or alkyl-O-C(O)-, wherein

R^{57} is hydrogen, or alkyl or phenyl optionally substituted with halogen.

Further embodiments of this embodiment are clear from the appended claims.

In one embodiment of the present invention, the compound is of the general formula (VII)



(VII)

wherein wherein

R^{27} is hydrogen or alkyl-O-CH₂-;

R^{28} and R^{29} independently of each other are hydrogen, halogen, dicyanovinyl, cyano, nitro, dinitrovinyl, alkyl optionally substituted with halogen, or aryl optionally substituted with one or more substituents selected from the group consisting of halogen, dicyanovinyl, cyano, nitro, and dinitrovinyl,

or

R^{28} and R^{29} together forms a benzene ring optionally substituted with one or more substituents selected from the group consisting of halogen, C₁₋₆-alkyl, dicyanovinyl, cyano, nitro, and dinitrovinyl;

and

R³⁰ is halogen, dicyanovinyl, cyano, nitro, dinitrovinyl, alkyl optionally substituted with halogen, or aryl optionally substituted with one or more substituents selected from the group consisting of halogen, dicyanovinyl, cyano, nitro, and dinitrovinyl; and R³¹ is hydrogen, halogen, dicyanovinyl, cyano, nitro, dinitrovinyl, alkyl optionally substituted with halogen, or aryl optionally substituted with one or more substituents selected from the group consisting of halogen, dicyanovinyl, cyano, nitro, and dinitrovinyl;

or

R³⁰ and R³¹ together forms a benzene ring optionally substituted with one or more substituents selected from the group consisting of halogen, C₁₋₆-alkyl, dicyanovinyl, cyano, nitro, and dinitrovinyl.

Further embodiments of this embodiment are clear from the appended claims.

In a use or a method according to the present invention, the compound may also be administered in combination with one or more further active substances in any suitable ratios. Such further active agents may be selected from antidiabetic agents, antihyperlipidemic agents, antiobesity agents, antihypertensive agents and agents for the treatment of complications resulting from or associated with diabetes.

Suitable antidiabetic agents include insulin, GLP-1 (glucagon like peptide-1) derivatives such as those disclosed in WO 98/08871 (Novo Nordisk A/S), which is incorporated herein by reference, as well as orally active hypoglycemic agents.

Suitable orally active hypoglycemic agents preferably include imidazolines, sulfonureas, biguanides, meglitinides, oxadiazolidinediones, thiazolidinediones, insulin sensitizers, α -glucosidase inhibitors, agents acting on the ATP-dependent potassium channel of the pancreatic β -cells eg potassium channel openers such as those disclosed in WO 97/26265, WO 99/03861 and WO 00/37474 (Novo Nordisk A/S) which are incorporated herein by reference, potassium channel openers, such as ormitiglinide, potassium channel blockers such as nateglinide or BTS-67582, glucagon antagonists such as those disclosed in WO 99/01423 and WO 00/39088 (Novo Nordisk A/S and Agouron Pharmaceuticals, Inc.), all of which are incorporated herein by reference, GLP-1 agonists such as those disclosed in WO 00/42026 (Novo Nordisk A/S and Agouron Pharmaceuticals, Inc.), which are incorporated herein by reference, DPP-IV (dipeptidyl peptidase-IV) inhibitors, PTPase (protein tyrosine phosphatase) inhibitors, glucokinase activators, such as those described in WO 02/08209 to Hoffmann La Roche, inhibitors of hepatic enzymes involved in stimulation of gluconeogenesis and/or glycogenolysis, glucose uptake modulators, GSK-3 (glycogen synthase kinase-3) inhibitors, compounds modifying the lipid metabolism such as antihyperlipidemic agents and

antilipidemic agents, compounds lowering food intake, and PPAR (peroxisome proliferator-activated receptor) and RXR (retinoid X receptor) agonists such as ALRT-268, LG-1268 or LG-1069.

5 In one embodiment of the uses and methods of the present invention, the compound involved may be administered in combination with insulin or insulin analogues.

In one embodiment of the uses and methods of the present invention, the compound involved may be administered in combination with a sulphonylurea eg tolbutamide, chlorpropamide, tolazamide, glibenclamide, glipizide, glimepiride, glicazide or glyburide.

10 In one embodiment of the uses and methods of the present invention, the compound involved may be administered in combination with a biguanide eg metformin.

In one embodiment of the uses and methods of the present invention, the compound involved may be administered in combination with a meglitinide eg repaglinide or senaglinide/nateglinide.

15 In one embodiment of the uses and methods of the present invention, the compound involved may be administered in combination with a thiazolidinedione insulin sensitizer eg troglitazone, ciglitazone, pioglitazone, rosiglitazone, isaglitazone, darglitazone, englitazone, CS-011/CI-1037 or T 174 or the compounds disclosed in WO 97/41097 (DRF-2344), WO 97/41119, WO 97/41120, WO 00/41121 and WO 98/45292 (Dr. Reddy's Research Foundation), which are incorporated herein by reference.

20 In one embodiment of the uses and methods of the present invention, the compound involved may be administered in combination with an insulin sensitizer eg such as GI 262570, YM-440, MCC-555, JTT-501, AR-H039242, KRP-297, GW-409544, CRE-16336, AR-H049020, LY510929, MBX-102, CLX-0940, GW-501516 or the compounds disclosed in WO 99/19313 (NN622/DRF-2725), WO 00/50414, WO 00/63191, WO 00/63192, WO
25 WO 00/63193 (Dr. Reddy's Research Foundation) and WO 00/23425, WO 00/23415, WO 00/23451, WO 00/23445, WO 00/23417, WO 00/23416, WO 00/63153, WO 00/63196, WO 00/63209, WO 00/63190 and WO 00/63189 (Novo Nordisk A/S), which are incorporated herein by reference.

30 In one embodiment of the uses and methods of the present invention, the compound involved may be administered in combination with an α -glucosidase inhibitor eg voglibose, emiglitate, miglitol or acarbose.

In one embodiment of the uses and methods of the present invention, the compound involved may be administered in combination with a glycogen phosphorylase inhibitor eg the compounds described in WO 97/09040 (Novo Nordisk A/S).

In one embodiment of the uses and methods of the present invention, the compound involved may be administered in combination with a glucokinase activator.

5 In one embodiment of the uses and methods of the present invention, the compound involved may be administered in combination with an agent acting on the ATP-dependent potassium channel of the pancreatic β -cells eg tolbutamide, glibenclamide, glipizide, glipizide, BTS-67582 or repaglinide.

In one embodiment of the uses and methods of the present invention, the compound involved may be administered in combination with nateglinide.

10 In one embodiment of the uses and methods of the present invention, the compound involved may be administered in combination with an antihyperlipidemic agent or a antilipidemic agent eg cholestyramine, colestipol, clofibrate, gemfibrozil, lovastatin, pravastatin, simvastatin, probucol or dextrothyroxine.

15 In one embodiment of the uses and methods of the present invention, the compound involved may be administered in combination with more than one of the above-mentioned compounds eg in combination with metformin and a sulphonylurea such as glyburide; a sulphonylurea and acarbose; nateglinide and metformin; acarbose and metformin; a sulphonylurea, metformin and troglitazone; insulin and a sulphonylurea; insulin and metformin; insulin, metformin and a sulphonylurea; insulin and troglitazone; insulin and lovastatin; etc.

20 In one embodiment of the uses and methods of the present invention, the compound involved may be administered in combination with one or more antiobesity agents or appetite regulating agents.

Such agents may be selected from the group consisting of CART (cocaine amphetamine regulated transcript) agonists, NPY (neuropeptide Y) antagonists, MC3 (melanocortin 3) agonists, MC4 (melanocortin 4) agonists, orexin antagonists, TNF (tumor necrosis factor) agonists, CRF (corticotropin releasing factor) agonists, CRF BP (corticotropin releasing factor binding protein) antagonists, urocortin agonists, β 3 adrenergic agonists such as CL-316243, AJ-9677, GW-0604, LY362884, LY377267 or AZ-40140, MSH (melanocyte-stimulating hormone) agonists, MCH (melanocyte-concentrating hormone) antagonists, CCK (cholecystokinin) agonists, serotonin reuptake inhibitors (fluoxetine, seroxat or citalopram),
25 serotonin and norepinephrine reuptake inhibitors, 5HT (serotonin) agonists, bombesin agonists, galanin antagonists, growth hormone, growth factors such as prolactin or placental lactogen, growth hormone releasing compounds, TRH (thyrotropin releasing hormone) agonists, UCP 2 or 3 (uncoupling protein 2 or 3) modulators, leptin agonists, DA (dopamine) agonists (bromocriptin, doprexin), lipase/amylase inhibitors, PPAR modulators, RXR modulators,
30 TR β agonists, adrenergic CNS stimulating agents, AGRP (agouti related protein) inhibi-

tors, H3 histamine antagonists such as those disclosed in WO 00/42023, WO 00/63208 and WO 00/64884, which are incorporated herein by reference, exendin-4, GLP-1 agonists and ciliary neurotrophic factor. Further antiobesity agents are bupropion (antidepressant), topiramate (anticonvulsant), ecopipam (dopamine D1/D5 antagonist), naltrexone (opioid antagonist), and peptide YY₃₋₃₆ (Batterham et al, Nature 418, 650-654 (2002)).

In one embodiment, the antiobesity agent is leptin.

In one embodiment, the antiobesity agent is peptide YY₃₋₃₆.

In one embodiment, the antiobesity agent is a serotonin and norepinephrine reuptake inhibitor eg sibutramine.

In one embodiment, the antiobesity agent is a lipase inhibitor eg orlistat.

In one embodiment, the antiobesity agent is an adrenergic CNS stimulating agent eg dexamphetamine, amphetamine, phentermine, mazindol phendimetrazine, diethylpropion, fenfluramine or dexfenfluramine.

Furthermore, in the uses and methods of the present invention, the compound involved may be administered in combination with one or more antihypertensive agents. Examples of antihypertensive agents are β -blockers such as alprenolol, atenolol, timolol, pindolol, propranolol and metoprolol, ACE (angiotensin converting enzyme) inhibitors such as benazepril, captopril, enalapril, fosinopril, lisinopril, quinapril and ramipril, calcium channel blockers such as nifedipine, felodipine, nicardipine, isradipine, nimodipine, diltiazem and verapamil, and α -blockers such as doxazosin, urapidil, prazosin and terazosin. Further reference can be made to Remington: The Science and Practice of Pharmacy, 19th Edition, Genaro, Ed., Mack Publishing Co., Easton, PA, 1995.

It should be understood that any suitable combination of the compounds according to the invention with diet and/or exercise, one or more of the above-mentioned compounds and optionally one or more other active substances are considered to be within the scope of the present invention.

The present invention also provides pharmaceutical compositions comprising as an active ingredient, at least one compound, preferably in a pharmacologically effective amount, more preferably in a therapeutically effective amount, suitable for any of the uses according to the present invention together with one or more pharmaceutically acceptable carriers or excipients.

The pharmaceutical composition is preferably in unit dosage form, comprising from about 0.05 mg to about 1000 mg, preferably from about 0.1 mg to about 500 mg and especially preferred from about 0.5 mg to about 200 mg of a compound suitable for any of the uses described above.

PHARMACEUTICAL COMPOSITIONS

The compounds for use according to the present invention may be administered alone or in combination with pharmaceutically acceptable carriers or excipients, in either single or multiple doses. The pharmaceutical compositions according to the invention may be formulated with pharmaceutically acceptable carriers or diluents as well as any other known adjuvants and excipients in accordance with conventional techniques such as those disclosed in Remington: The Science and Practice of Pharmacy, 19th Edition, Gennaro, Ed., Mack Publishing Co., Easton, PA, 1995.

The pharmaceutical compositions may be specifically formulated for administration by any suitable route such as the oral, rectal, nasal, pulmonary, topical (including buccal and sublingual), transdermal, intracisternal, intraperitoneal, vaginal and parenteral (including subcutaneous, intramuscular, intrathecal, intravenous and intradermal) route, the oral route being preferred. It will be appreciated that the preferred route will depend on the general condition and age of the subject to be treated, the nature of the condition to be treated and the active ingredient chosen.

Pharmaceutical compositions for oral administration include solid dosage forms such as hard or soft capsules, tablets, troches, dragees, pills, lozenges, powders and granules. Where appropriate, they can be prepared with coatings such as enteric coatings or they can be formulated so as to provide controlled release of the active ingredient such as sustained or prolonged release according to methods well known in the art.

Liquid dosage forms for oral administration include solutions, emulsions, aqueous or oily suspensions, syrups and elixirs.

Pharmaceutical compositions for parenteral administration include sterile aqueous and non-aqueous injectable solutions, dispersions, suspensions or emulsions as well as sterile powders to be reconstituted in sterile injectable solutions or dispersions prior to use. Depot injectable formulations are also contemplated as being within the scope of the present invention.

Other suitable administration forms include suppositories, sprays, ointments, cremes, gels, inhalants, dermal patches, implants etc.

A typical oral dosage is in the range of from about 0.001 to about 100 mg/kg body weight per day, preferably from about 0.01 to about 50 mg/kg body weight per day, and more preferred from about 0.05 to about 10 mg/kg body weight per day administered in one or more dosages such as 1 to 3 dosages. The exact dosage will depend upon the frequency and mode of administration, the sex, age, weight and general condition of the subject

treated, the nature and severity of the condition treated and any concomitant diseases to be treated and other factors evident to those skilled in the art.

The formulations may conveniently be presented in unit dosage form by methods known to those skilled in the art. A typical unit dosage form for oral administration one or
5 more times per day such as 1 to 3 times per day may contain from 0.05 to about 1000 mg, preferably from about 0.1 to about 500 mg, and more preferred from about 0.5 mg to about 200 mg.

For parenteral routes such as intravenous, intrathecal, intramuscular and similar ad-
10 ministration, typically doses are in the order of about half the dose employed for oral administration.

The present invention also encompasses pharmaceutically acceptable salts of the compounds suitable for use according to the present invention. Such salts include pharmaceutically acceptable acid addition salts, pharmaceutically acceptable metal salts, ammonium and alkylated ammonium salts. Acid addition salts include salts of inorganic acids as well as
15 organic acids. Representative examples of suitable inorganic acids include hydrochloric, hydrobromic, hydroiodic, phosphoric, sulfuric, nitric acids and the like. Representative examples of suitable organic acids include formic, acetic, trichloroacetic, trifluoroacetic, propionic, benzoic, cinnamic, citric, fumaric, glycolic, lactic, maleic, malic, malonic, mandelic, oxalic, picric, pyruvic, salicylic, succinic, methanesulfonic, ethanesulfonic, tartaric, ascorbic, pantoic,
20 bismethylene salicylic, ethanedisulfonic, gluconic, citraconic, aspartic, stearic, palmitic, EDTA, glycolic, p-aminobenzoic, glutamic, benzenesulfonic, p-toluenesulfonic acids and the like. Further examples of pharmaceutically acceptable inorganic or organic acid addition salts include the pharmaceutically acceptable salts listed in J. Pharm. Sci. 1977, 66, 2, which is incorporated herein by reference. Examples of metal salts include lithium, sodium, potassium, magnesium salts and the like. Examples of ammonium and alkylated ammonium salts include ammonium, methylammonium, dimethylammonium, trimethylammonium, ethylammonium, hydroxyethylammonium, diethylammonium, butylammonium, tetramethylammonium salts and the like.

Also intended as pharmaceutically acceptable acid addition salts are the hydrates
30 which the present compounds are able to form.

The compounds for use according to the present invention are generally utilized as the free substance or as a pharmaceutically acceptable salt thereof. Examples are an acid addition salt of a compound having the utility of a free base and a base addition salt of a compound having the utility of a free acid. The term "pharmaceutically acceptable salts" refers to non-toxic
35 salts of the compounds for use according to the present invention which salts are generally

prepared by reacting the free base with a suitable organic or inorganic acid or by reacting the acid with a suitable organic or inorganic base. When a compound for use according to the present invention contains a free base such salts are prepared in a conventional manner by treating a solution or suspension of the compound with a chemical equivalent of a pharmaceutically acceptable acid. When a compound for use according to the present invention, contains a free acid such salts are prepared in a conventional manner by treating a solution or suspension of the compound with a chemical equivalent of a pharmaceutically acceptable base. Physiologically acceptable salts of a compound with a hydroxy group include the anion of said compound in combination with a suitable cation such as sodium or ammonium ion. Other salts which are not pharmaceutically acceptable may be useful in the preparation of compounds of the invention and these form a further aspect of the invention.

For parenteral administration, solutions of the compounds for use according to the present invention in sterile aqueous solution, aqueous propylene glycol or sesame or peanut oil may be employed. Such aqueous solutions should be suitably buffered if necessary and the liquid diluent first rendered isotonic with sufficient saline or glucose. The aqueous solutions are particularly suitable for intravenous, intramuscular, subcutaneous and intraperitoneal administration. The sterile aqueous media employed are all readily available by standard techniques known to those skilled in the art.

Suitable pharmaceutical carriers include inert solid diluents or fillers, sterile aqueous solution and various organic solvents. Examples of solid carriers are lactose, terra alba, sucrose, cyclodextrin, talc, gelatine, agar, pectin, acacia, magnesium stearate, stearic acid and lower alkyl ethers of cellulose. Examples of liquid carriers are syrup, peanut oil, olive oil, phospholipids, fatty acids, fatty acid amines, polyoxyethylene and water. Similarly, the carrier or diluent may include any sustained release material known in the art, such as glyceryl monostearate or glyceryl distearate, alone or mixed with a wax. The pharmaceutical compositions formed by combining the compounds for use according to the present invention and the pharmaceutically acceptable carriers are then readily administered in a variety of dosage forms suitable for the disclosed routes of administration. The formulations may conveniently be presented in unit dosage form by methods known in the art of pharmacy.

Formulations of the present invention suitable for oral administration may be presented as discrete units such as capsules or tablets, each containing a predetermined amount of the active ingredient, and which may include a suitable excipient. Furthermore, the orally available formulations may be in the form of a powder or granules, a solution or suspension in an aqueous or non-aqueous liquid, or an oil-in-water or water-in-oil liquid emulsion.

Compositions intended for oral use may be prepared according to any known method, and such compositions may contain one or more agents selected from the group consisting of sweetening agents, flavouring agents, colouring agents, and preserving agents in order to provide pharmaceutically elegant and palatable preparations. Tablets may contain the active ingredient in admixture with non-toxic pharmaceutically-acceptable excipients which are suitable for the manufacture of tablets. These excipients may be for example, inert diluents, such as calcium carbonate, sodium carbonate, lactose, calcium phosphate or sodium phosphate; granulating and disintegrating agents, for example corn starch or alginic acid; binding agents, for example, starch, gelatine or acacia; and lubricating agents, for example magnesium stearate, stearic acid or talc. The tablets may be uncoated or they may be coated by known techniques to delay disintegration and absorption in the gastrointestinal tract and thereby provide a sustained action over a longer period. For example, a time delay material such as glyceryl monostearate or glyceryl distearate may be employed. They may also be coated by the techniques described in U.S. Patent Nos. 4,356,108; 4,166,452; and 4,265,874, incorporated herein by reference, to form osmotic therapeutic tablets for controlled release.

Formulations for oral use may also be presented as hard gelatine capsules where the active ingredient is mixed with an inert solid diluent, for example, calcium carbonate, calcium phosphate or kaolin, or a soft gelatine capsules wherein the active ingredient is mixed with water or an oil medium, for example peanut oil, liquid paraffin, or olive oil.

Aqueous suspensions may contain the compound for use according to the present invention in admixture with excipients suitable for the manufacture of aqueous suspensions. Such excipients are suspending agents, for example sodium carboxymethylcellulose, methylcellulose, hydroxypropylmethylcellulose, sodium alginate, polyvinylpyrrolidone, gum tragacanth and gum acacia; dispersing or wetting agents may be a naturally-occurring phosphatide such as lecithin, or condensation products of an alkylene oxide with fatty acids, for example polyoxyethylene stearate, or condensation products of ethylene oxide with long chain aliphatic alcohols, for example, heptadecaethyl-eneoxycetanol, or condensation products of ethylene oxide with partial esters derived from fatty acids and a hexitol such as polyoxyethylene sorbitol monooleate, or condensation products of ethylene oxide with partial esters derived from fatty acids and hexitol anhydrides, for example polyethylene sorbitan monooleate. The aqueous suspensions may also contain one or more colouring agents, one or more flavouring agents, and one or more sweetening agents, such as sucrose or saccharin.

Oily suspensions may be formulated by suspending the active ingredient in a vegetable oil, for example arachis oil, olive oil, sesame oil or coconut oil, or in a mineral oil such as a liquid paraffin. The oily suspensions may contain a thickening agent, for example beeswax, hard paraffin or cetyl alcohol. Sweetening agents such as those set forth above, and
5 flavouring agents may be added to provide a palatable oral preparation. These compositions may be preserved by the addition of an anti-oxidant such as ascorbic acid.

Dispersible powders and granules suitable for preparation of an aqueous suspension by the addition of water provide the active compound in admixture with a dispersing or wetting agent, suspending agent and one or more preservatives. Suitable dispersing or wetting agents and suspending agents are exemplified by those already mentioned above. Additional excipients, for example, sweetening, flavouring, and colouring agents may also be present.
10

The pharmaceutical compositions comprising compounds for use according to the present invention may also be in the form of oil-in-water emulsions. The oily phase may be a vegetable oil, for example, olive oil or arachis oil, or a mineral oil, for example a liquid paraffin, or a mixture thereof. Suitable emulsifying agents may be naturally-occurring gums, for example gum acacia or gum tragacanth, naturally-occurring phosphatides, for example soy bean, lecithin, and esters or partial esters derived from fatty acids and hexitol anhydrides, for example sorbitan monooleate, and condensation products of said partial esters with ethylene oxide, for example polyoxyethylene sorbitan monooleate. The emulsions may also contain
15 20 sweetening and flavouring agents.

Syrups and elixirs may be formulated with sweetening agents, for example glycerol, propylene glycol, sorbitol or sucrose. Such formulations may also contain a demulcent, a preservative and flavouring and colouring agents. The pharmaceutical compositions may be
25 in the form of a sterile injectable aqueous or oleaginous suspension. This suspension may be formulated according to the known methods using suitable dispersing or wetting agents and suspending agents described above. The sterile injectable preparation may also be a sterile injectable solution or suspension in a non-toxic parenterally-acceptable diluent or solvent, for example as a solution in 1,3-butanediol. Among the acceptable vehicles and solvents that
30 may be employed are water, Ringer's solution, and isotonic sodium chloride solution. In addition, sterile, fixed oils are conveniently employed as solvent or suspending medium. For this purpose, any bland fixed oil may be employed using synthetic mono- or diglycerides. In addition, fatty acids such as oleic acid find use in the preparation of injectables.

The compositions may also be in the form of suppositories for rectal administration
35 of the compounds of the invention. These compositions can be prepared by mixing the drug

with a suitable non-irritating excipient which is solid at ordinary temperatures but liquid at the rectal temperature and will thus melt in the rectum to release the drug. Such materials include cocoa butter and polyethylene glycols, for example.

For topical use, creams, ointments, jellies, solutions or suspensions, etc., containing the compounds of the invention are contemplated. For the purpose of this application, topical applications shall include mouth washes and gargles.

The compounds of the present invention may also be administered in the form of liposome delivery systems, such as small unilamellar vesicles, large unilamellar vesicles, and multilamellar vesicles. Liposomes may be formed from a variety of phospholipids, such as cholesterol, stearylamine, or phosphatidylcholines.

In addition, some of the compounds of the present invention may form solvates with water or common organic solvents. Such solvates are also encompassed within the scope of the invention.

Thus, in a further embodiment, there is provided a pharmaceutical composition comprising a compound for use according to the present invention, or a pharmaceutically acceptable salt, solvate, or prodrug thereof, and one or more pharmaceutically acceptable carriers, excipients, or diluents.

If a solid carrier is used for oral administration, the preparation may be tableted, placed in a hard gelatine capsule in powder or pellet form or it can be in the form of a troche or lozenge. The amount of solid carrier will vary widely but will usually be from about 25 mg to about 1 g. If a liquid carrier is used, the preparation may be in the form of a syrup, emulsion, soft gelatine capsule or sterile injectable liquid such as an aqueous or non-aqueous liquid suspension or solution.

A typical tablet that may be prepared by conventional tableting techniques may contain:

Core:

Active compound (as free compound or salt thereof)	5.0 mg
Lactosum Ph. Eur.	67.8 mg
Cellulose, microcryst. (Avicel)	31.4 mg
Amberlite®IRP88*	1.0 mg
Magnesii stearas Ph. Eur.	q.s.

Coating:

Hydroxypropyl methylcellulose	approx.	9 mg
Mywacett 9-40 T**	approx.	0.9 mg

* Polacrillin potassium NF, tablet disintegrant, Rohm and Haas.

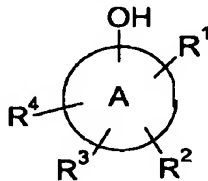
** Acylated monoglyceride used as plasticizer for film coating.

5 If desired, the pharmaceutical composition comprising a compound for use according to the present invention may comprise a compound for use according to the present invention in combination with further active substances such as those described in the foregoing.

The present invention also provides methods for the preparation of compounds for use according to the present invention. The compounds can be prepared readily according to
10 the following general procedures (in which all variables are as defined before, unless so specified) using readily available starting materials, reagents and conventional synthesis procedures. In these reactions, it is also possible to make use of variants which are themselves known to those of ordinary skill in this art, but are not mentioned in greater detail.

GENERAL PROCEDURES

15 **General procedure (A): Preparation of compounds of the general formula I**

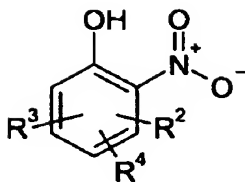


(I)

20 Compounds of the general formula (I), wherein A, R¹, R², R³, and R⁴ are as described above, are aryl or heteroaryl derivatives with electron withdrawing groups that make the compounds of formula (I) more acidic than the corresponding unsubstituted phenolic aryl or heteroaryl.

The compounds of formula (I) may be prepared from the corresponding phenolic aryls or heteroaryl by e.g. nitration of the corresponding phenols. The resulting nitrophenols may be converted to many halogenated derivatives by conversion to aniline, diazotization or
25 by other methods known to the person skilled in the art. Other methods of substituting phenolic aryls and heteroaryl with substituents like R¹, R², R³, and R⁴ are known to the person skilled in the art

44

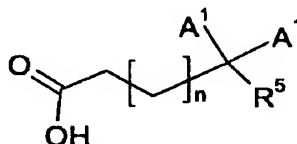


(Ia)

Compounds of the general formula (Ia), a subgroup of the compounds of general formula (I), wherein R^2 , R^3 , and R^4 are as described above, are phenol derivatives with electro withdrawing groups that make the compounds of formula (Ia) more acidic than phenol.

The compounds of formula (Ia) may be prepared from the corresponding phenols by treatment with a mixture of sulphuric acid and nitric acid by methods which are known by those skilled in the art and described in *Vogels Textbook of practical organic chemistry* (5th ed Longman Scientific and Technical, 1989).

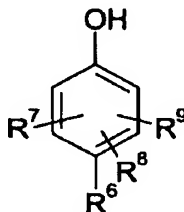
10 General procedure (B)



(II)

Compounds of the general formula (II), wherein A^1 , A^2 , R^5 , and n are as described above, may be prepared from bis-phenolic aryls or heteroaryl by nitration. Bis-phenolic aryls or heteroaryl are available by a number of routes, including treatment of aldehydes, ketones or alkenes with phenol in the presence of suitable catalysts or in protected versions with Grignard reagents treated with ketones, aldehydes or esters.

General procedure (C)

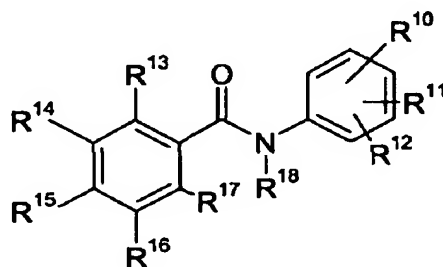


(III)

Compounds of the general formula (III), wherein R^6 , R^7 , R^8 , and R^9 are as described above, are phenol derivatives with electro withdrawing groups that make the compounds of formula (Ia) more acidic than phenol.

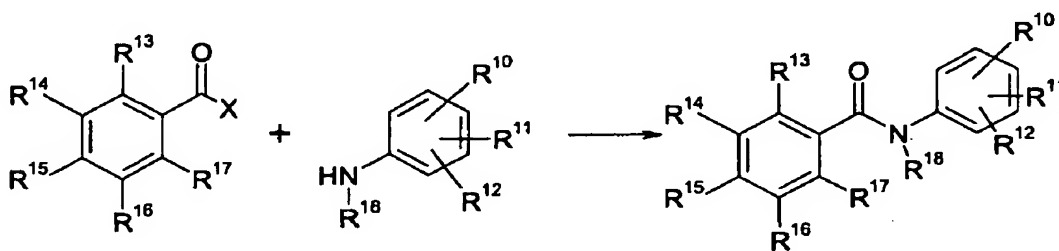
- 5 The compounds of formula (III) may be prepared from the corresponding phenols by treatment with a mixture of sulphuric acid and nitric acid by methods which are known by those skilled in the art and described in *Vogels Textbook of practical organic chemistry*.

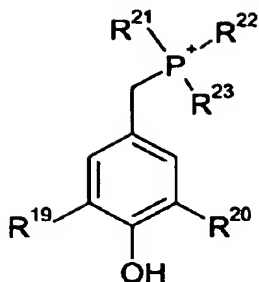
General procedure (D)



(IV)

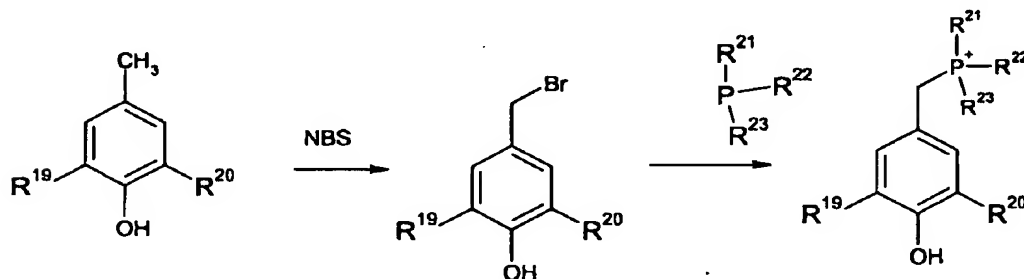
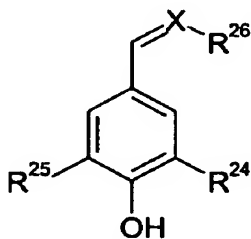
- 10 Compounds of the general formula (IV), wherein R^{10} , R^{11} , R^{12} , R^{13} , R^{14} , R^{15} , R^{16} , R^{17} , and R^{18} are as described above, may be prepared from a substituted benzoic acid ($X=OH$) or a benzoyl chloride ($X=Cl$). In the former case reagents such phosphorous trichloride, phosphorous pentachloride, phosphorous tribromide or oxalyl chloride is employed and solvents such as collidine, chlorobenzene or toluene. The methods are described in e.g. Brown et. al.
- 15 (J. Med. Chem. 1985, 143-146).



General procedure (E)

(V)

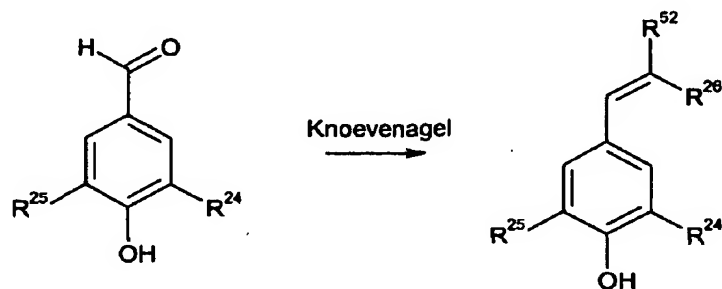
Compounds of general formula (V), wherein R^{19} , R^{20} , R^{21} , R^{22} , and R^{23} are as described above, may be prepared by treating commercially available 4-methylphenols with N-bromosuccinimide and a radical initiator such as benzoylperoxide in a solvent such as carbon tetrachloride for 1 to 24 hours under reflux. The 4-bromomethylphenols produced this way is treated with a trialkylphosphine, such as tripropylphosphine, or a trialkylphosphine, such as triphenylphosphine, in a solvent such as tetrahydrofuran or acetone. The salt can eventually be precipitated with a solvent such as diethylether or hexane.

**General procedure (F)**

(VI)

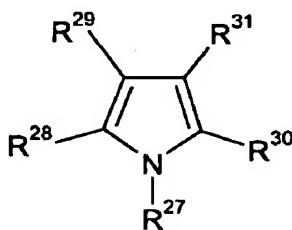
Compounds of the general formula (VI), wherein R^{24} , R^{25} , and R^{26} are as described above and X is $=C(R^{52})-$, wherein R^{52} are as described above, may be prepared from the corresponding aldehyde which may be commercially available (e.g. 3,5-di-tert-butyl-4-

hydroxybenzaldehyde) or which can be made by reactions known to the person skilled in the art, such as exemplified in Vogels "Textbook of organic chemistry", and which aldehyde by a Knoevenagel reaction with a suitable substrate such as malonodinitrile can be made to react to generate compounds of the general formula (VI) by heating in a solvent such as toluene or benzene with ammonium acetate as catalyst and isolation by methods known by those skilled in the art.



Compounds of the general formula (VI), wherein R^{24} , R^{25} , and R^{26} are as described above and X is =N-, may be prepared from the corresponding aldehyde and a commercially available primary amine such as exemplified in Vogels "Textbook of organic chemistry".

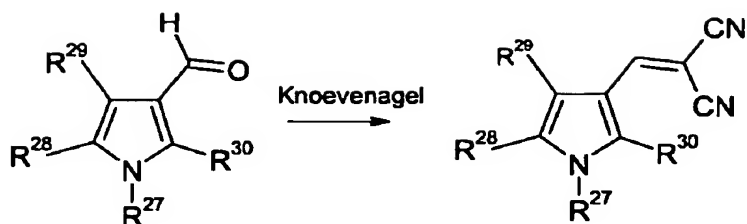
General procedure (G)



(VII)

Compounds of general formula (VII), wherein R^{27} , R^{28} , R^{29} , R^{30} , and R^{31} are as described above, may be prepared by introducing R^{31} via the corresponding aldehyde, which may be commercially available (e.g. indole-3-carboxaldehyde) or which can be made by reactions known to the person skilled in the art, such as exemplified in Vogels Textbook of practical organic chemistry and which aldehyde by a Knoevenagel reaction with a suitable substrate such as malononitrile can be made to react to generate compounds of formula (VII) by heating in a solvent such as toluene or benzene with ammonium acetate as catalyst and isolation by methods known by those skilled in the art. Alternatively, the electro withdrawing group R^{31} may be present in the ring forming step using methods described in Eicher et al. "The chemistry of heterocycles" (Thieme, 1995, 1 ed.) and references herein.

48



Such compounds may also be prepared by classical pyrrole syntheses such as the Hantzsch and Knorr procedures (see "The chemistry of Heterocycles" Eicher et al, Thieme) or by the methods described in Kuhn et al. *Phytochemicals for Pest Control*, ACS Symposium Series 658, 194-205 (1997), and Kuhn et al, *Pesticide Science* 41, 279-286 (1994).

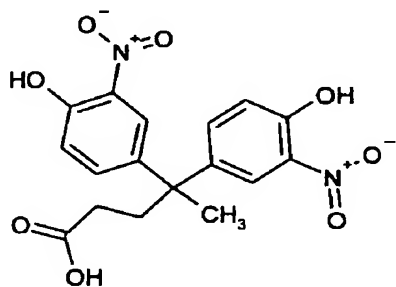
5

The following examples show compounds for use as a chemical uncoupler as described herein as well as comparative examples showing comparative compounds.

EXAMPLES

Example 1 (General procedure (B))

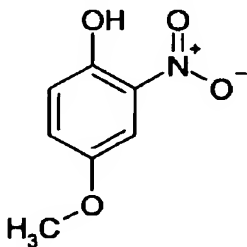
10 4,4-Bis-(4-hydroxy-3-nitrophenyl)-valeric acid



The title compound may be prepared according to General procedure B or purchased from Aldrich Chemical Company, Inc., catalogue number F209216.

Example 2 (General procedure (A))

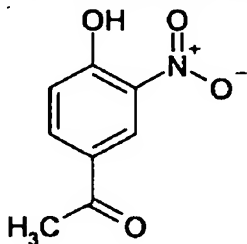
15 4-Methoxy-2-nitrophenol



The title compound may be prepared according to General procedure A or purchased from Aldrich Chemical Company, Inc., catalogue number 1568-70-3.

Example 3 (General procedure (A))

4-Hydroxy-3-nitroacetophenone

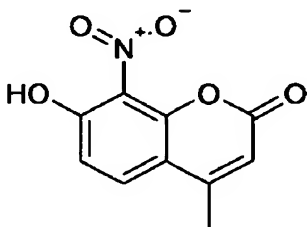


5

The title compound may be prepared according to General procedure A or purchased from Aldrich Chemical Company, Inc., catalogue number 6622-56-1.

Example 4 (General procedure (A))

7-Hydroxy-4-methyl-8-nitro-chromen-2-one

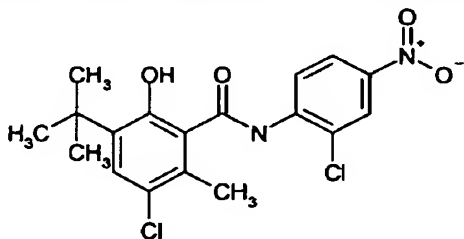


10

The title compound may be prepared according to General procedure A or purchased from Lancaster Synthesis Ltd., catalogue number F7896.

Example 5 (General procedure (D))

3-Tert-butyl-5-chloro-N-(2-chloro-4-nitrophenyl)-2-hydroxy-6-methyl-benzamide

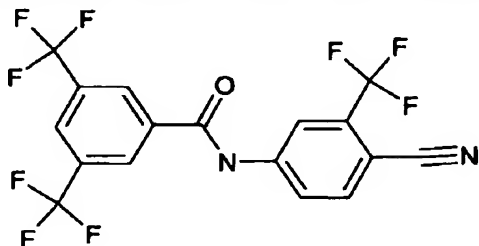


15

The title compound may be prepared according to General procedure D or purchased from Sigma-Aldrich Chemie GmbH, catalogue number R548758.

Example 6 (General procedure (D))

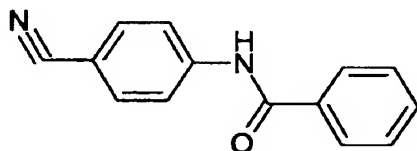
N-1-[4-cyano-3-(trifluoromethyl)phenyl]-3,5-di(trifluoromethyl)benzamide



The title compound may be prepared according to General procedure D or purchased from Maybridge plc, catalogue number RDR03708.

Example 7 (General procedure (D))

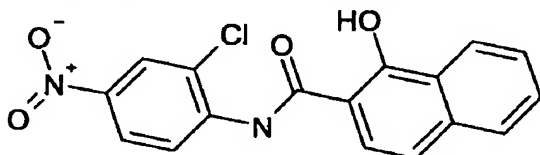
N-(4-cyanophenyl)benzamide



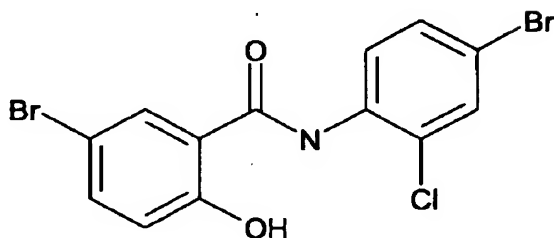
The title compound may be prepared according to General procedure D or purchased from Maybridge plc, catalogue number HTS05127.

Example 8 (General procedure (D))

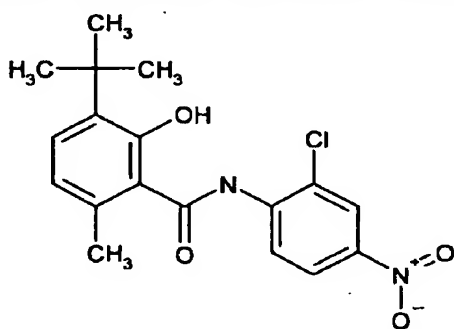
2'-Chloro-1-hydroxy-4'-nitro-2-naphthanilide



The title compound may be prepared according to General procedure D or purchased from Aldrich Chemical Company, Inc., catalogue number S62,938-3.

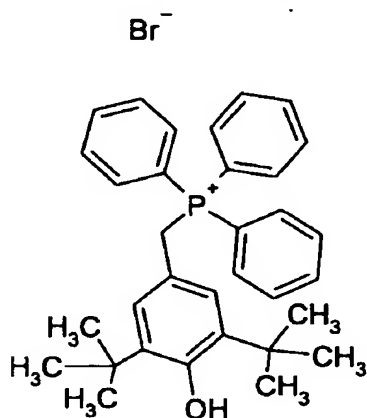
Example 9 (General procedure (D))**N-(2-chloro-4-bromophenyl)-5-bromosalicylanilide**

The title compound may be prepared according to General procedure D or purchased from Maybridge plc, catalogue number BTB 12821.

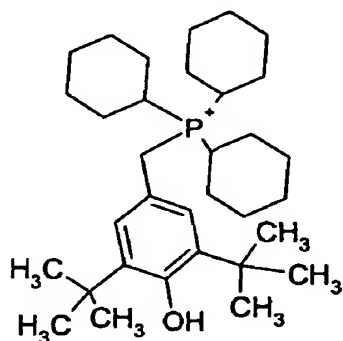
Example 10 (General procedure (D))**N-(2-chloro-4-nitrophenyl)-3-tert-butyl-6-methylsalicylanilide**

3-tert-Butyl-6-methylsalicylic acid (5.0 g, 24 mmol) and 2-chlor-4-nitroanilin (4.1 g; 24 mmol) were dissolved in chlorobenzene (140 ml). Phosphoroxychloride (0.63 ml) was added and the mixture was refluxed 4.5 hours under nitrogen atmosphere. The solvent was removed in vacuo and the residue crystallized from acetic acid yielding 6.3 g of the title compound.

¹H-NMR: (CDCl₃, 400 MHz): 1.43 (s, 9H); 2.65 (s, 3H); 6.76 (d, 1H); 7.34 (d, 1H); 8.22 (dd, 1H); 8.42 (d, 1H); 8.47 (s (br); 1H); 8.86 (d, 1H); 10.3 (s (br); 1H).

Example 11 (General procedure (E))**(3,5-Di-*tert*-butyl-4-hydroxybenzyl)triphenylphosphonium bromide**

The title compound may be prepared according to General procedure E or purchased from Sigma-Aldrich Chemie GmbH, catalogue number S545813.

Example 12 (General procedure (E))**(3,5-Di-*tert*-butyl-4-hydroxybenzyl)tricyclohexylphosphonium bromide**

The title compound may be prepared according to General procedure E.

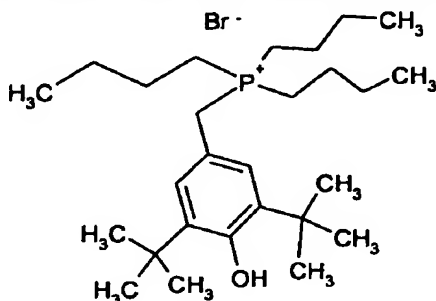
2,6-Di-*tert*-butyl-4-methylphenol is treated with 1 equivalent of N-bromo-succinimide in tetrachloromethane under reflux for 1 hour in the presence of 0.1 equivalent of benzoylperoxide. After evaporation of the solvent, 2,6-di-*tert*-butyl-4-bromomethylphenol is isolated using gradient elution on silica gel with heptane/methylene chloride as eluent. The crude 2,6-di-*tert*-butyl-4-bromomethylphenol (1 equivalent) is dissolved in dry diethyl ether (10% solution) and treated with a 10% solution of trialkyl- or triarylphosphine in diethyl ether. After 1 hour of stir-

ring, crystalline material is filtered off.

LCMS m/z : 499.5 ($M+H$)⁺

Example 13 (General procedure (E))

(3,5-Di-*tert*-butyl-4-hydroxybenzyl)tributylphosphonium bromide



5

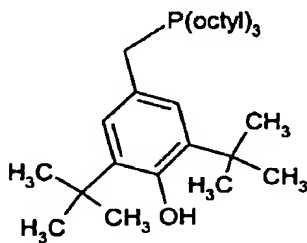
The title compound may be prepared according to General procedure E.

2,6-Di-*tert*-butyl-4-methylphenol is treated with 1 equivalent of N-bromo-succinimide in tetra-chloromethane under reflux for 1 hour in the presence of 0.1 equivalent of benzoylperoxide. After evaporation of the solvent, 2,6-di-*tert*-butyl-4-bromomethylphenol is isolated using gra-
 10 dent elution on silica gel with heptane/methylene chloride as eluent. The crude 2,6-di-*tert*-butyl-4-bromomethylphenol (1 equivalent) is dissolved in dry diethyl ether (10% solution) and treated with a 10% solution of tributylphosphine in diethyl ether. After 1 hour of stirring, crys-
 talline material is filtered off.

LCMS m/z : 421.3 ($M+H$)⁺

15 Example 14 (General procedure (E))

(3,5-Di-*tert*-butyl-4-hydroxybenzyl)trioctylphosphonium bromide



The title compound may be prepared according to General procedure E.

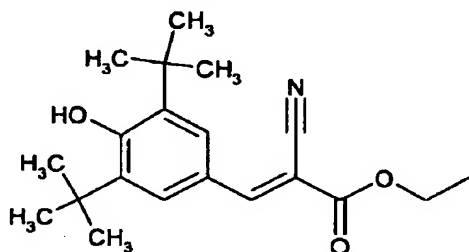
2,6-Di-*tert*-butyl-4-methylphenol is treated with 1 equivalent of N-bromo-succinimide in tetra-
 20 chloromethane under reflux for 1 hour in the presence of 0.1 equivalent of benzoylperoxide. After evaporation of the solvent, 2,6-di-*tert*-butyl-4-bromomethylphenol is isolated using gra-

dient elution on silica gel with heptane/methylene chloride as eluent. The crude 2,6-di-*tert*-butyl-4-bromomethylphenol (1 equivalent) is dissolved in dry diethyl ether (10% solution) and treated with a 10% solution of trioctylphosphine in diethyl ether. After 1 hour of stirring, the ether is decanted off, the oil triturated with ether and the remaining oil is dried *in vacuo*.

5 LCMS m/z : 591.0 ($M+H$)⁺

Example 15 (General procedure (F))

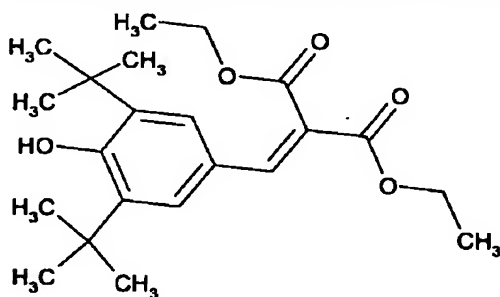
2-Cyano-3-(3,5-di-*tert*-butyl-4-hydroxyphenyl)-acrylic acid ethyl ester



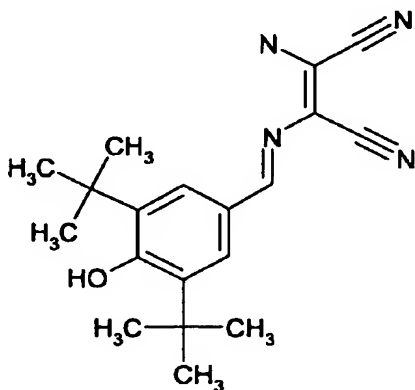
10 The title compound may be prepared according to General procedure F or purchased from Sigma-Aldrich Chemie GmbH, catalogue number S482102.

Example 16 (General procedure (F))

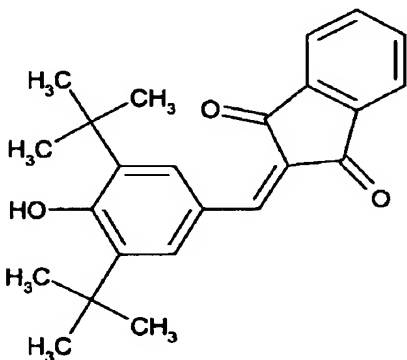
2-(3,5-Di-*tert*-butyl-4-hydroxy-benzylidene)-malonic acid diethyl ester



15 The title compound may be prepared according to General procedure F or purchased from Sigma-Aldrich Chemie GmbH, catalogue number S482196.

Example 17 (General procedure (F))**2-Amino-S-[(3,5-di-tert-butyl-4-hydroxybenzylidene)-amino]-but-2-enedinitrile**

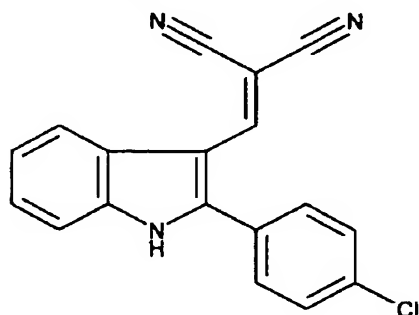
The title compound may be purchased from Menai, catalogue number DW 150.

5 Example 18 (General procedure (F))**2-(3,5-Di-tert-butyl-4-hydroxy-benzylidene)-indan-1,3-dione**

The title compound may be prepared according to General procedure F or purchased from Maybridge plc, catalogue number LJ 902.

Example 19 (General procedure (G))

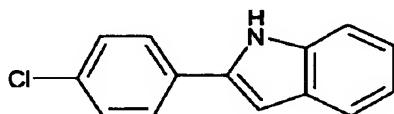
2-[[2-(4-Chlorophenyl)-1H-indol-3-yl]methylene]malononitrile



The title compound may be prepared according to General procedure G or purchased from Maybridge plc, catalogue number SEW 03041.

Example 20 (General procedure (G))

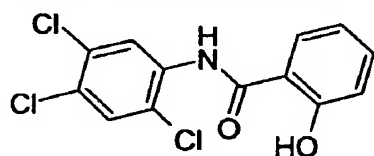
2-(4-Chlorophenyl)-indole



The title compound may be prepared according to General procedure G or purchased from Maybridge plc, catalogue number RDR 01154.

Example 21

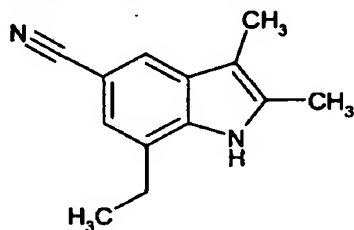
N-(2,4,5-trichlorophenyl)salicylanilide



The title compound may purchased from Maybridge plc, catalogue number RDR 01398.

Example 22 (General procedure (G))

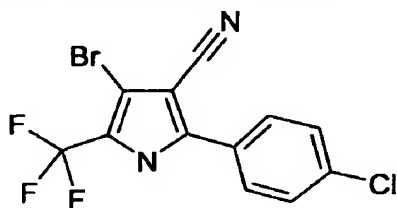
2,3-Dimethyl-5-cyano-7-ethylindole



The title compound may be purchased from Aldrich Chemical Company, Inc., catalogue number 30,205-8.

Example 23 (General procedure (G))

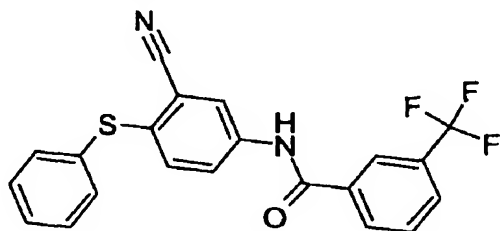
4-Bromo-2-(4-chlorophenyl)-5-trifluoromethyl-1H-pyrrole-3-carbonitrile



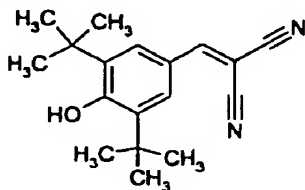
This compound was prepared as described in Kameshwaran, Synthesis 5, 530 (1997).

Example 24

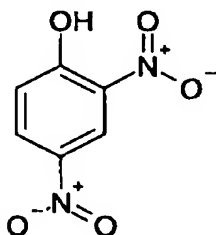
N-(3-Cyano-4-phenylsulfanyl-phenyl)-3-trifluoromethyl-benzamide



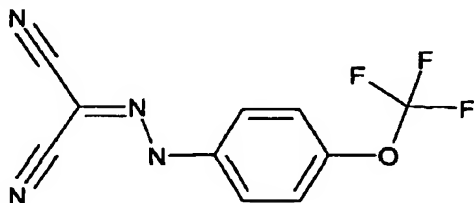
This compound is commercially available from Bionet (cat nr. 11K-627S).

Example 25**2,6-Di-*t*-butyl-4-(2',2'-dicyanovinyl)phenol**

The title compound may be prepared according to General procedure F or purchased from Biomol research Laboratories Inc., catalogue number EI-215.

Comparative example 26**2,4-Dinitrophenol**

Available from Sigma-Aldrich Chemie GmbH, catalogue number D-7004.

10 Comparative Example 27**Carbonylcyanide *p*-trifluoromethoxy-phenylhydrazone**

Available from Sigma-Aldrich Chemie GmbH, catalogue number F-86,184-7.

PHARMACOLOGICAL METHODS**15 Assay (I): Glucose utilisation in a human hepatocytes cell line (HEP-G2 cells)****Assay description:**

The assay measures indirectly the activity of the respiratory chain in HEP-G2 cells by using D-(5-³H(N))-glucose. The ³H-proton will be released during the metabolism of glucose where it will be incorporated into water. The water is thereafter separated from the D-(5-

$^3\text{H}(\text{N})$ -glucose by evaporation. Finally, the radioactivity in the water is determined using a Topcounter.

Method:

HEP-G2 cells obtained from ATCC (Maryland, USA), are cultured in growth medium (McCoy's 5A, 5ml Pen/Strep and 10% FCS). The assay medium used in this assay is (MEM medium) with the following addition 1x non-essential amino acids (M7145, 2 mM glutamine, 100 units/ml penicillin and streptomycin, 0.0075% sodium bicarbonate, 1 mM sodium pyruvate and 0.5% BSA (Bovine Serum Albumin, Sigma Missouri, USA)) at 37°C and 5% CO_2 . All media are obtained by Gibco (Life Technologies, Maryland, USA) where not otherwise mentioned.

At day zero the cells are harvested using trypsin-EDTA and washed in assay medium using centrifugation. The cells are plated into single StripPlates wells (Corning B.V. Life Sciences, The Netherlands) that are placed into 24-well plates (Corning B.V. Life Sciences, The Netherlands) with a concentration of 2×10^4 cells/100 μl /well. The cells are then incubated at 37°C and 5% CO_2 overnight.

The next day the compounds to be tested are diluted in DMSO (Sigma, Missouri, USA) to 100 times final concentration. They are then diluted to a final concentration in assay medium containing 10 $\mu\text{Ci/ml}$ D-(5- $^3\text{H}(\text{N})$)-glucose (PerkinElmer Life Sciences Inc., Boston, USA). The medium is removed from the cells and 200 μl of the compound dilutions are added in duplicates. The cells are then incubated for another 3 hours at 37°C and 5% CO_2 . Finally the cells are lysed by adding 50 μl 10% TCA (trichloroacetate). 300 μl of sterile water is then added to the 24-wells that surrounds the StripPlate wells. The plate is sealed with Top-seal-tape (Packard, PerkinElmer Life Sciences Inc., Boston, USA) and the plate is incubated in a heating cupboard at 50°C to equilibrium the radioactive water formed in the respiratory chain into the water in the 24-well plate by evaporate. The plates incubate for 8 hours where the heating cupboard is turned off. The top seal is removed when the samples have reached room temperature. One ml scintillation liquid (Packard Microscint, PerkinElmer Life Sciences Inc., Boston, USA) is added to all the samples and the radioactivity is determined using a Topcounter (Packard, PerkinElmer Life Sciences Inc., Boston, USA). Non-specific activity is determined by evaporating 200 μl of the dilution medium containing the D-(5- $^3\text{H}(\text{N})$)-glucose into 300 μl sterile water, and total radioactivity is determined by counting 5 μl assay medium with 10 $\mu\text{Ci/ml}$ D-(5- $^3\text{H}(\text{N})$)-glucose.

Calculations:

The cpm (counts per minute) representing the non-specific radioactivity in the D-(5- $^3\text{H}(\text{N})$)-glucose is subtracted from all incubated values. Then mean basal value (samples

without any compound added) is subtracted from the values of all stimulated samples (samples added different concentrations of the different test compounds). All these calculations are done using GraphPad Prism 3.0 (GraphPad software, Inc.). The maximal stimulation caused by the test compounds is calculated in percentage of maximal stimulation caused by either DNP or FCCP. If a test compound represents a saturated maximal stimulation that is less than 75% of maximal DNP/FCCP stimulation, that is, E_{max} for the test compound is less than 75% of the E_{max} for DNP or FCCP in Assay (I), it is considered as a partial stimulator, see figure 1, wherein the curve for SF6847 is compared to the curve for a partial stimulator according to the present invention. An E_{max} of less than 75% of the E_{max} for DNP or FCCP may signify that a saturation of the uncoupling process is taking place.

When calculating the concentration-response curve the maximal glucose utilisation stimulated by FCCP or DNP (subtracted non-specific and basal values) is converted to 100% and all the other values are expressed in percentage of this value. Using these percentage values and the compound concentration a concentration-response curve is calculated using sigmoidal dose-response (variable slope) equation (all the calculations are done by GraphPad Prism 3.0 (GraphPad software, Inc.));

Equation 1:

$$Y = \text{Bottom} + (\text{Top} - \text{Bottom}) / (1 + 10^{((\text{LogEC}_{50} - X) \cdot \text{slope})})$$

X is the logarithm of the molar concentration of the test compound.

Y is the degree of stimulation caused by the compound measured as a percentage of the maximal stimulation achieved by use of FCCP or DNP.

Y starts at Bottom which is the value for the stimulation caused by the lowest concentration of the test compound and goes to Top which is the value for the stimulation caused by the highest concentration of the test compound and the curve for Y as a function of X has a sigmoid shape. The concentration of the test compound that stimulates the glucose utilisation with 50% is defined as EC_{50} . From the calculated EC_{50} value, new concentrations of the compound corresponding to $3 \times EC_{50}$, $2 \times EC_{50}$, $EC_{50}/2$, $EC_{50}/3$ and control are tested in Assay (I). The results achieved with these concentrations are used to determine the slope in this area using the following equation:

Equation 2:

$$(Y_2 - Y_0) / (Y_1 - Y_0) = X^n$$

where Y_0 is the degree of stimulation measured as counts per minute (cpm) in Assay (I) in control samples without added test compound, Y_1 is the degree of stimulation measured as cpm in Assay (I) with added test compound in a concentration of either $EC_{50}/2$ or $EC_{50}/3$, and Y_2 is the degree of stimulation measured as cpm in Assay (I) with added test compound in concentration of either $2xEC_{50}$ or $3xEC_{50}$, X is either 2 or 3, and n is the slope.

The equation should be understood such that when Y_1 is the degree of stimulation with added test compound in a concentration of $EC_{50}/2$, then Y_2 is the degree of stimulation with added test compound in concentration of $2xEC_{50}$, and X is 2, and when Y_1 is the degree of stimulation with added test compound in a concentration of $EC_{50}/3$, then Y_2 is the degree of stimulation with added test compound in a concentration of $3xEC_{50}$, and X is 3.

The slope calculated by this equation is defined as the change in response divided with the change in test compound concentration around the EC_{50} value of the compound. If this equation is high then a small change in test compound concentration cause a great change in the response, like when using DNP or FCCP. If the slope is close to unity then a small change in test compound concentration cause a small change in the response, a relation that can be used to determine that the test compound has a broader safety-window than DNP or FCCP. This slope may thus be used to describe safe chemical uncouplers for use according to the present invention (see figure 2).

Another way of describing safe chemical uncouplers is that no sign of toxicity (defined as a significant drop in glucose utilisation in Assay (I)) may occur within a ten fold increase of the concentration of the compound needed for achieving E_{max} . This characteristic will for the purpose of this application be designated a "long E_{max} ", meaning that for compounds with a long E_{max} , the glucose utilisation does not decrease for concentrations ranging from the concentration of the compound needed for achieving E_{max} to 10 times this concentration. Compounds with a long E_{max} may be regarded as safe chemical uncouplers

The following Table 1 shows the data from Assay (I) for the compounds of the examples.

Table 1

Ex	E_{max} (% of FCCP)	CE_{max}	Slope	DiE	EC_{50} (μ m)
1	45	100	1.5	-	40
2	35	1000	-	-	290
3	75	1000	-	-	270

Ex	E _{max} (% of FCCP)	CE _{max}	Slope	DiE	EC ₅₀ (μ m)
4	70	1000	1.4	-	150
5	95	0.3	1.7	"Long E _{max} "	0.06
6	95	10	-	-	1.6
7	45	1000	0.83	-	180
8	80	100	-	-	12
9	100	10	3.1	"Long E _{max} "	2.3
10	100	3	1.7	"Long E _{max} "	0.37
11	75	3	-	>100	0.7
12	70	10	-	>30	1.2
13	70	10	-	>30	2.9
14	100	10	-	>10	1.1
15	100	3	-	"Long E _{max} "	0.7
16	100	100	2.3	-	11
17	45	30	-	>100	6.0
18	80	3	-	"Long E _{max} "	0.6
19	100	100	1.53	-	12
20	55	300	1.0	-	50
21	100	30	3.3	-	7
22	100	1000	-	-	91
23	115	30	-	"Long E _{max} "	2
24	65	300	2.3	-	42
25	100	10	1.3	"Long E _{max} "	0.48
26	100	500	3.0	>500	225
27	100	30	2.2	"Long E _{max} "	3.2

Ex

= Example number

E_{max} (% of FCCP)= E_{max} calculated as described in Assay (I): Glucose utilisation in a human hepatocytes cell line (HEP-G2 cells)

and given in percentages of the E_{max} of FCCP in the same assay.

CE_{max}

= the concentration of the compound at which E_{max} is achieved

5 Slope

= mean of slope calculated from equation 2

DiE (Decline in Efficacy)

= DiE indicates the concentration of the compound at which a beginning decrease in glucose utilisation, as described above, is observed. "Long E_{max} " indicates that a decrease in glucose utilisation is not observed at a concentration of the compound of more than 10 times the concentration achieving E_{max} . Values given as for instance >500 μM indicates that no decrease in glucose utilisation is observed at a concentration of compound of 500 μM or below, but at higher concentrations. See for instance Figure 3.

10

15

EC_{50}

= EC_{50} calculated as described in Assay (I): Glucose utilisation in a human hepatocytes cell line (HEP-G2 cells)

20 Assay (II) - The effect of chemical uncouplers on mitochondrial respiration using isolated mitochondria.

Assay description:

This assay is used to investigate if the increase in glucose utilisation caused by the test compounds observed in the glucose utilisation assay is due to an increase in the respiration of the mitochondria. This is done by measuring oxygen consumption in isolated mitochondria.

25

A Clark oxygen electrode is used to determine the oxygen consumption. The isolated mitochondria are added to medium containing oxygen and nutrients (e.g. Succinate) and the rate of oxygen consumption is measured, when stabilized a small amount of ADP is added to increase the respiration and an increase in the rate of oxygen consumption is measured. When the rate of oxygen consumption again has stabilized the test compound is added and a further increase in oxygen consumption is seen. This experiment is done with and without the addition of oligomycin which is an inhibitor of the ATP-synthase. Finally, the

30

test compound is examined without adding ADP. If the test compound stimulates the rate of oxygen consumption in all setups, it is regarded as a chemical uncoupler.

Assay (III): Identification of chemical uncouplers (full or partial chemical uncouplers) that increase energy expenditure *in vivo*

- 5 The effect of the chemical uncouplers (full or partial agonists) on energy expenditure (oxygen consumption) *in vivo* is determined by indirect calorimetry. Briefly, animals are placed in air-tight chambers. Air is continuously led to and from the chambers. The gas concentrations of oxygen (O₂) and carbondioxide (CO₂) in the air led to and from the chambers (inlet and outlet air) are recorded and the consumption of O₂ and the production of CO₂ are calculated. Based
10 on the amount of O₂ consumed and CO₂ produced, energy expenditure is calculated. Compounds which at a given dose increase whole body energy expenditure without obvious deleterious effects are deemed to be chemical uncouplers that increase energy expenditure.

Assay (IV): Glucose utilisation in FSK-4 cells

15 **Assay description:**

- The assay measures indirectly the activity of the respiratory chain in FSK-4 cells by using D-(6-³H(N))-glucose. The ³H-proton will first be released in the TCA cyclus and transported to the respiratory chain where it will be incorporated into water. The water is thereafter separated from the D-(6-³H(N))-glucose by evaporation. Finally, the radioactivity in the water
20 is determined using a Topcounter.

Method:

- FSK-4 cells obtained from ATCC (Maryland, USA), are cultured in growth medium (McCoy's medium with the following addition 100 units/ml penicillin and streptomycin and 10 % FCS (fetal calf serum)) at 37°C and 5% CO₂. All media are obtained by Gibco (Life Technologies, Maryland, USA) where not otherwise mentioned.
25

- At day zero the cells are harvested using trypsin-EDTA and washed in assay medium (MEM medium with the following addition 1x non-essential amino acids (M7145, 2 mM glutamin, 100 units/ml pencillin and streptomycin, 0.0075% sodium bicarbonate, 1 mM sodium pyrovate and 2 % horse serum) using centrifugation. The cells are plated into single
30 StripPlates wells (Corning B.V.Life Sciences, The Netherlands) that are placed into 24-well plates (Corning B.V.Life Sciences, The Netherlands) with a concentration of 1,5x10⁴ cells/100 µl assay medium/well. The cells are then incubated at 37°C and 5% CO₂ overnight.

- The next day the compounds to be tested are diluted to different concentrations in DMSO (Sigma, Missouri, USA) to 100 times final concentration. They are then diluted to a final concentration in assay medium containing 10 $\mu\text{Ci/ml}$ D-(6- $^3\text{H}(\text{N})$)-glucose (PerkinElmer Life Sciences Inc., Boston, USA). The medium is removed from the cells and 200 μl of the compound dilutions are added in duplicates. The cells are then incubated for another 24 hours at 37°C and 5% CO_2 . Finally the cells are lysed by adding 50 μl 10% TCA (tri-chloroacetate). 300 μl of sterile water is then added to the 24-wells that surrounds the Strip-Plate wells. The plate is sealed with Top-seal-tape (Packard, PerkinElmer Life Sciences Inc., Boston, USA) and the plate is incubated in a heating cupboard at 50°C to equilibrium the radioactive water formed in the respiratory chain into the water in the 24-well plate by evaporation. The plates incubate for 8 hours where the heating cupboard is turned off. The top seal is removed when the samples have reached room temperature. One ml scintillation liquid (Packard Microscient, PerkinElmer Life Sciences Inc., Boston, USA) is added to all the samples and the radioactivity is determined using a Topcounter (Packard, PerkinElmer Life Sciences Inc., Boston, USA). Non-specific activity is determined by evaporating 200 μl of the dilution medium containing the D-(6- $^3\text{H}(\text{N})$)-glucose into 300 μl sterile water, and total radioactivity is determined by counting 5 μl assay medium with 10 $\mu\text{Ci/ml}$ D-(6- $^3\text{H}(\text{N})$)-glucose.

Calculations:

- All calculations are done using GraphPad Prism 3.0 (GraphPad software, Inc.)
From concentration-response curves the half maximal concentration (EC_{50}) and maximal efficacy (E_{max}) are calculated using equation 1.

Equation 1:

$$Y = \text{Bottom} + (\text{Top} - \text{Bottom}) / (1 + 10^{((\text{LogEC}_{50} - X) \cdot \text{slope})})$$

X is the logarithm of the molar concentration of the test compound.

Y is the degree of stimulation caused by the compound measured as percentage of the basal stimulation.

- Y starts at Bottom which is the value for the stimulation caused by the lowest concentration of the test compound and goes to Top which is the value for the stimulation caused by the highest concentration of the test compound and the curve for Y as a function of X has a sigmoid shape.

The calculated EC_{50} value is then used to determine the concentrations used in a study where the Hill slope is calculated. In the Hill slope study concentrations between 0.05 to 10 times EC_{50} are used.

From the double logarithmic plot of the increase in glucose utilisation and the compound concentration the Hill slope is calculated accordingly to equation 2.

Equation 2:

$$\frac{V}{V_{\max}} = \frac{[X]^n}{K_H + [X]^n} \rightleftharpoons$$

$$\frac{V_{\max}}{V} = \frac{K_H}{[X]^n} + 1 \rightleftharpoons$$

$$\frac{V_{\max} - V}{V} = \frac{K_H}{[X]^n} \rightleftharpoons$$

$$\text{Log } V_{\max} - \text{Log } V = \text{Log } K_H - n \cdot \text{Log } [X] \rightleftharpoons$$

$$\text{Log } V = \text{Log } V_{\max} - \text{Log } K_H + n \cdot \text{Log } [X]$$

X is the molar concentration of the test compound

V is the increase in glucose utilisation

V_{\max} is the theoretic maximal no-limiting increase in glucose utilisation

K_H is the Hill equation constant

n is the Hill slope

It is presumed, that the maximal increase in the glucose utilisation (as measured in the FSK-4 glucose utilisation assay) is limited by the capacity of the metabolism. It is furthermore presumed, that the glucose utilisation would reach a much higher degree of stimulation if the metabolism were not the limiting factor (the theoretical maximal stimulation in the FSK-4 glucose utilisation if metabolism were not the limiting factor is denoted V_{\max} in the equation above). Consequently, $V_{\max} - V \approx V_{\max}$, where V is the measured increase in FSK-4 glucose utilisation. Finally, it is assumed that $\text{Log } V_{\max} - \text{Log } K_H$ is a constant.

CLAIMS

1. Use of a compound, with an E_{\max} in Assay (I), as described in the specification, of less than 75% of the E_{\max} of carbonylcyanide *p*-trifluoromethoxy-phenylhydrazine in Assay (I), or a pharmaceutically acceptable salt, solvate or prodrug thereof, for increasing mitochondrial respiration.
5
2. Use of a compound with an E_{\max} in Assay (I), as described in the specification, of less than 75% of the E_{\max} of carbonylcyanide *p*-trifluoromethoxy-phenylhydrazine in Assay (I), or a pharmaceutically acceptable salt, solvate or prodrug thereof, for treating a condition benefiting from an increase in mitochondrial respiration in a patient in need thereof.
10
3. Use of a compound with an E_{\max} in Assay (I), as described in the specification, of less than 75% of the E_{\max} of carbonylcyanide *p*-trifluoromethoxy-phenylhydrazine in Assay (I), or a pharmaceutically acceptable salt, solvate or prodrug thereof, for the preparation of a pharmaceutical composition for treating a condition benefiting from an increase in mitochondrial respiration in a patient in need thereof.
15
4. Use according to claim 2 or claim 3, where the condition to be treated is a condition selected from obesity, atherosclerosis, hypertension, diabetes, type 2 diabetes, impaired glucose tolerance, dyslipidemia, coronary heart disease, gallbladder disease, osteoarthritis, and cancers such as endometrial, breast, prostate and colon cancers in a patient in need thereof.
20
5. Use according to claim 4, wherein the condition is obesity.
- 25 6. Use according to claim 4, wherein the disease is type 2 diabetes.
7. Use according to claim 6, wherein the patient in need thereof is obese.
8. Use according to claim 4, wherein the disease is dyslipidemia.
30
9. Use according to claim 8, wherein the patient in need thereof is obese.
10. Use of a compound, with an E_{\max} in Assay (I), as described in the specification, of less than 75% of the E_{\max} of carbonylcyanide *p*-trifluoromethoxy-phenylhydrazine in Assay (I), or

a pharmaceutically acceptable salt, solvate or prodrug thereof, for reducing reactive oxygen species.

- 5 11. Use of a compound with an E_{\max} in Assay (I), as described in the specification, of less than 75% of the E_{\max} of carbonylcyanide *p*-trifluoromethoxy-phenylhydrazone in Assay (I), or a pharmaceutically acceptable salt, solvate or prodrug thereof, for treating a condition benefiting from a reduction of reactive oxygen species in a patient in need thereof.
- 10 12. Use of a compound with an E_{\max} in Assay (I), as described in the specification, of less than 75% of the E_{\max} of carbonylcyanide *p*-trifluoromethoxy-phenylhydrazone in Assay (I), or a pharmaceutically acceptable salt, solvate or prodrug thereof, for the preparation of a pharmaceutical composition for treating a condition benefiting from a reduction of reactive oxygen species in a patient in need thereof.
- 15 13. Use according to claim 11 or claim 12, wherein the condition to be treated is the aging process, damage of heart tissue, damage of neuronal tissue, Alzheimer's disease, cancer, or cataract.
- 20 14. Use of a compound with an E_{\max} in Assay (I), as described in the specification, of less than 75% of the E_{\max} of 2,4-dinitrophenol in Assay (I), or a pharmaceutically acceptable salt, solvate or prodrug thereof, for increasing mitochondrial respiration.
- 25 15. Use of a compound with an E_{\max} in Assay (I), as described in the specification, of less than 75% of the E_{\max} of 2,4-dinitrophenol in Assay (I), or a pharmaceutically acceptable salt, solvate or prodrug thereof, for treating a condition benefiting from an increase in mitochondrial respiration in a patient in need thereof.
- 30 16. Use of a compound with an E_{\max} in Assay (I), as described in the specification, of less than 75% of the E_{\max} of 2,4-dinitrophenol in Assay (I), or a pharmaceutically acceptable salt, solvate or prodrug thereof, for the preparation of a pharmaceutical composition for treating a condition benefiting from an increase in mitochondrial respiration in a patient in need thereof.
17. Use according to claim 15 or claim 16, where the condition to be treated is a condition selected from obesity, atherosclerosis, hypertension, diabetes, type 2 diabetes, impaired glu-

cose tolerance, dyslipidemia, coronary heart disease, gallbladder disease, osteoarthritis, and cancers such as endometrial, breast, prostate and colon cancers in a patient in need thereof.

18. Use according to claim 17, wherein the condition is obesity.

5

19. Use according to claim 17, wherein the disease is type 2 diabetes.

20. Use according to claim 19, wherein the patient in need thereof is obese.

10

21. Use according to claim 17, wherein the disease is dyslipidemia.

22. Use according to claim 21, wherein the patient in need thereof is obese.

15

23. Use of a compound with an E_{\max} in Assay (I), as described in the specification, of less than 75% of the E_{\max} of 2,4-dinitrophenol in Assay (I), or a pharmaceutically acceptable salt, solvate or prodrug thereof, for reducing reactive oxygen species.

20

24. Use of a compound with an E_{\max} in Assay (I), as described in the specification, of less than 75% of the E_{\max} of 2,4-dinitrophenol in Assay (I), or a pharmaceutically acceptable salt, solvate or prodrug thereof, for treating a condition benefiting from a reduction of reactive oxygen species in a patient in need thereof.

25

25. Use of a compound with an E_{\max} in Assay (I), as described in the specification, of less than 75% of the E_{\max} of 2,4-dinitrophenol in Assay (I), or a pharmaceutically acceptable salt, solvate or prodrug thereof, for the preparation of a pharmaceutical composition for treating a condition benefiting from a reduction of reactive oxygen species in a patient in need thereof.

30

26. Use according to claim 24 or claim 25, wherein the condition to be treated is the aging process, damage of heart tissue, damage of neuronal tissue, Alzheimer's disease, cancer, or cataract.

27. Use of a compound with a slope calculated from the equation

$$X^n = (Y_2 - Y_0) / (Y_1 - Y_0)$$

wherein

Y_0 is the degree of stimulation measured as counts per minute (cpm) in Assay (I) in control samples without added test compound,

and

Y_1 is the degree of stimulation measured as cpm in Assay (I) with added test compound in a concentration of $EC_{50}/2$,

Y_2 is the degree of stimulation measured as cpm in Assay (I) with added test compound in concentration of $2xEC_{50}$, and

X is 2,

or

Y_1 is the degree of stimulation measured as cpm in Assay (I) with added test compound in a concentration of $EC_{50}/3$,

Y_2 is the degree of stimulation measured as cpm in Assay (I) with added test compound in concentration of $3xEC_{50}$, and

X is 3,

and

n is the slope,

of a value equal to or less than the value for the slope calculated from the above equation with carbonylcyanide *p*-trifluoromethoxy-phenylhydrazone as test compound in Assay (I), or a pharmaceutically acceptable salt, solvate or prodrug thereof, for increasing mitochondrial respiration.

28. Use of a compound with a slope calculated from the equation

$$X^n = (Y_2 - Y_0) / (Y_1 - Y_0)$$

wherein

Y_0 is the degree of stimulation measured as counts per minute (cpm) in Assay (I) in control samples without added test compound,

and

Y_1 is the degree of stimulation measured as cpm in Assay (I) with added test compound in a concentration of $EC_{50}/2$,

Y_2 is the degree of stimulation measured as cpm in Assay (I) with added test compound in concentration of $2xEC_{50}$, and

X is 2,

or

Y_1 is the degree of stimulation measured as cpm in Assay (I) with added test compound in a concentration of $EC_{50}/3$,

Y_2 is the degree of stimulation measured as cpm in Assay (I) with added test compound in concentration of $3xEC_{50}$, and

X is 3,

and

5

n is the slope,

of a value equal to or less than the value for the slope calculated from the above equation with carbonylcyanide *p*-trifluoromethoxy-phenylhydrazone as test compound in Assay (I), or a pharmaceutically acceptable salt, solvate or prodrug thereof, for treating a condition benefiting from an increase in mitochondrial respiration in a patient in need thereof.

10

29. Use of a compound with a slope calculated from the equation

$$X^n = (Y_2 - Y_0) / (Y_1 - Y_0) = X^n$$

wherein

15

Y_0 is the degree of stimulation measured as counts per minute (cpm) in Assay (I) in control samples without added test compound,

and

Y_1 is the degree of stimulation measured as cpm in Assay (I) with added test compound in a concentration of $EC_{50}/2$,

20

Y_2 is the degree of stimulation measured as cpm in Assay (I) with added test compound in concentration of $2xEC_{50}$, and

X is 2,

or

Y_1 is the degree of stimulation measured as cpm in Assay (I) with added test compound in a concentration of $EC_{50}/3$,

25

Y_2 is the degree of stimulation measured as cpm in Assay (I) with added test compound in concentration of $3xEC_{50}$, and

X is 3,

and

n is the slope,

30

of a value equal to or less than the value for the slope calculated from the above equation with carbonylcyanide *p*-trifluoromethoxy-phenylhydrazone as test compound in Assay (I), or a pharmaceutically acceptable salt, solvate or prodrug thereof, for the preparation of a pharmaceutical composition for treating a condition benefiting from an increase in mitochondrial respiration in a patient in need thereof.

35

30. Use according to claim 28 or claim 29, where the condition to be treated is a condition selected from obesity, atherosclerosis, hypertension, diabetes, type 2 diabetes, impaired glucose tolerance, dyslipidemia, coronary heart disease, gallbladder disease, osteoarthritis, and cancers such as endometrial, breast, prostate and colon cancers in a patient in need thereof.

5

31. Use according to claim 30, wherein the condition is obesity.

32. Use according to claim 30, wherein the disease is type 2 diabetes.

10

33. Use according to claim 32, wherein the patient in need thereof is obese.

34. Use according to claim 30, wherein the disease is dyslipidemia.

35. Use according to claim 34, wherein the patient in need thereof is obese.

15

36. Use of a compound with a slope calculated from the equation

$$X^n = (Y_2 - Y_0) / (Y_1 - Y_0) = X^n$$

wherein

20

Y_0 is the degree of stimulation measured as counts per minute (cpm) in Assay (I) in control samples without added test compound,

and

Y_1 is the degree of stimulation measured as cpm in Assay (I) with added test compound in a concentration of $EC_{50}/2$,

Y_2 is the degree of stimulation measured as cpm in Assay (I) with added test compound in concentration of $2 \times EC_{50}$, and

25

X is 2,

or

Y_1 is the degree of stimulation measured as cpm in Assay (I) with added test compound in a concentration of $EC_{50}/3$,

30

Y_2 is the degree of stimulation measured as cpm in Assay (I) with added test compound in concentration of $3 \times EC_{50}$, and

X is 3,

and

n is the slope,

of a value equal to or less than the value for the slope calculated from the above equation with carbonylcyanide *p*-trifluoromethoxy-phenylhydrazone as test compound in Assay (I), or a pharmaceutically acceptable salt, solvate or prodrug thereof, for reducing reactive oxygen species.

5

37. Use of a compound with a slope calculated from the equation

$$X^n = (Y_2 - Y_0) / (Y_1 - Y_0)$$

wherein

Y_0 is the degree of stimulation measured as counts per minute (cpm) in Assay (I) in control samples without added test compound,

10

and

Y_1 is the degree of stimulation measured as cpm in Assay (I) with added test compound in a concentration of $EC_{50}/2$,

Y_2 is the degree of stimulation measured as cpm in Assay (I) with added test compound in concentration of $2 \times EC_{50}$, and

15

X is 2,

or

Y_1 is the degree of stimulation measured as cpm in Assay (I) with added test compound in a concentration of $EC_{50}/3$,

20

Y_2 is the degree of stimulation measured as cpm in Assay (I) with added test compound in concentration of $3 \times EC_{50}$, and

X is 3,

and

n is the slope,

25

of a value equal to or less than the value for the slope calculated from the above equation with carbonylcyanide *p*-trifluoromethoxy-phenylhydrazone as test compound in Assay (I), or a pharmaceutically acceptable salt, solvate or prodrug thereof, for treating a condition benefiting from a reduction of reactive oxygen species in a patient in need thereof.

30

38. Use of a compound with a slope calculated from the equation

$$X^n = (Y_2 - Y_0) / (Y_1 - Y_0)$$

wherein

Y_0 is the degree of stimulation measured as counts per minute (cpm) in Assay (I) in control samples without added test compound,

35

and.

Y_1 is the degree of stimulation measured as cpm in Assay (I) with added test compound in a concentration of $EC_{50}/2$,

Y_2 is the degree of stimulation measured as cpm in Assay (I) with added test compound in concentration of $2xEC_{50}$, and

5 X is 2,

or

Y_1 is the degree of stimulation measured as cpm in Assay (I) with added test compound in a concentration of $EC_{50}/3$,

10 Y_2 is the degree of stimulation measured as cpm in Assay (I) with added test compound in concentration of $3xEC_{50}$, and

X is 3,

and

n is the slope,

15 of a value equal to or less than the value for the slope calculated from the above equation with carbonylcyanide *p*-trifluoromethoxy-phenylhydrazone as test compound in Assay (I), or a pharmaceutically acceptable salt, solvate or prodrug thereof, for the preparation of a pharmaceutical composition for treating a condition benefiting from a reduction of reactive oxygen species in a patient in need thereof.

20 39. Use according to claim 37 or claim 38, wherein the condition to be treated is the aging process, damage of heart tissue, damage of neuronal tissue, Alzheimer's disease, cancer, or cataract.

25 40. Use according to any of claims 27 to 39, wherein the value for the slope calculated from the equation

$$X^n = (Y_2 - Y_0) / (Y_1 - Y_0)$$

is significantly less than the value for the slope calculated with carbonylcyanide *p*-trifluoromethoxy-phenylhydrazone as test compound in Assay (I).

30 41. Use of a compound with a Hill slope, n, calculated as described in Assay IV in the description, equal to or lower than said slope calculated for carbonylcyanide *p*-trifluoromethoxy-phenylhydrazone, or a pharmaceutical acceptable salt, solvate or prodrug of said compound, for increasing mitochondrial respiration.

42. Use of a compound with a Hill slope, n , calculated as described in Assay IV in the description, equal to or lower than said slope calculated for carbonylcyanide *p*-trifluomethoxyphenylhydrazone, or a pharmaceutical acceptable salt, solvate or prodrug of said compound, for treating conditions benefiting from an increase in mitochondrial respiration.

5

43. Use of a compound with a Hill slope, n , calculated as described in Assay IV in the description, equal to or lower than said slope calculated for carbonylcyanide *p*-trifluomethoxyphenylhydrazone, or a pharmaceutical acceptable salt, solvate or prodrug of said compound, for the preparation of a pharmaceutical composition for treating a condition benefiting from an increase in mitochondrial respiration.

10

44. Use according to claim 42 or claim 43, where the condition to be treated is a condition selected from obesity, atherosclerosis, hypertension, diabetes, type 2 diabetes, impaired glucose tolerance, dyslipidemia, coronary heart disease, gallbladder disease, osteoarthritis, and cancers such as endometrial, breast, prostate and colon cancers.

15

45. The use according to 44, wherein the condition is obesity.

46. The use according to 44, wherein the condition is type 2 diabetes.

20

47. The use according to 46, wherein the patient is obese.

48. The use according to 44, wherein the condition is dyslipidemia.

25

49. The use according to 48, wherein the patient is obese.

50. Use of a compound with a Hill slope, n , calculated as described in Assay IV in the description, equal to or lower than said slope calculated for carbonylcyanide *p*-trifluomethoxyphenylhydrazone, or a pharmaceutical acceptable salt, solvate or prodrug of said compound, for reducing reactive oxygen species.

30

51. Use of a compound with a Hill slope, n , calculated as described in Assay IV in the description, equal to or lower than said slope calculated for carbonylcyanide *p*-trifluomethoxyphenylhydrazone, or a pharmaceutical acceptable salt, solvate or prodrug of said compound, for treating diseases benefiting from a reduction in reactive oxygen species.

35

52. Use of a compound with a Hill slope, n , calculated as described in Assay IV in the description, equal to or lower than said slope calculated for carbonylcyanide *p*-trifluoromethoxy-phenylhydrazone, or a pharmaceutical acceptable salt, solvate or prodrug of said compound,
5 in the preparation of a pharmaceutical composition for treating a condition benefiting from a reduction in reactive oxygen species.

53. The use according to claim 51 or claim 52, wherein the condition to be treated is the aging process, damage of heart tissue, damage of neuronal tissue, Alzheimer's disease, cancer,
10 or cataract.

54. The use accruing to any of claims 41 to 53, wherein the Hill slope, n , as calculated in Assay IV in the description is significantly less than said slope calculated for carbonylcyanide *p*-trifluoromethoxy-phenylhydrazone.
15

55. Use of a compound for which the value for the glucose utilisation caused by the compound in Assay (I) in concentrations of from CE_{max} to ten times CE_{max} does not fall significantly below the value of E_{max} for the compound in Assay (I), or a pharmaceutically acceptable salt, solvate or prodrug thereof, for increasing mitochondrial respiration.
20

56. Use of a compound for which the value for the glucose utilisation caused by the compound in Assay (I) in concentrations of from CE_{max} to ten times CE_{max} does not fall significantly below the value of E_{max} for the compound in Assay (I), or a pharmaceutically acceptable salt, solvate or prodrug thereof, for treating a condition benefiting from an increase in mitochondrial respiration in a patient in need thereof.
25

57. Use of a compound for which the value for the glucose utilisation caused by the compound in Assay (I) in concentrations of from CE_{max} to ten times CE_{max} does not fall significantly below the value of E_{max} for the compound in Assay (I), or a pharmaceutically acceptable salt, solvate or prodrug thereof, for the preparation of a pharmaceutical composition for treating a condition benefiting from an increase in mitochondrial respiration in a patient in need thereof.
30

58. Use according to claim 56 or claim 57, where the condition to be treated is a condition selected from obesity, atherosclerosis, hypertension, diabetes, type 2 diabetes, impaired glu-
35

cose tolerance, dyslipidemia, coronary heart disease, gallbladder disease, osteoarthritis, and cancers such as endometrial, breast, prostate and colon cancers in a patient in need thereof.

59. Use according to claim 58, wherein the condition is obesity.

5

60. Use according to claim 58, wherein the disease is type 2 diabetes.

61. Use according to claim 60, wherein the patient in need thereof is obese.

10 62. Use according to claim 58, wherein the disease is dyslipidemia.

63. Use according to claim 62, wherein the condition to be treated is the aging process, damage of heart tissue, damage of neuronal tissue, Alzheimer's disease, cancer, or cataract.

15 64. Use of a compound for which the value for the glucose utilisation caused by the compound in Assay (I) in concentrations of from CE_{max} to ten times CE_{max} does not fall significantly below the value of E_{max} for the compound in Assay (I), or a pharmaceutically acceptable salt, solvate or prodrug thereof, for reducing reactive oxygen species.

20 65. Use of a compound for which the value for the glucose utilisation caused by the compound in Assay (I) in concentrations of from CE_{max} to ten times CE_{max} does not fall significantly below the value of E_{max} for the compound in Assay (I), or a pharmaceutically acceptable salt, solvate or prodrug thereof, for treating a condition benefiting from a reduction of reactive oxygen species in a patient in need thereof.

25

66. Use of a compound for which the value for the glucose utilisation caused by the compound in Assay (I) in concentrations of from CE_{max} to ten times CE_{max} does not fall significantly below the value of E_{max} for the compound in Assay (I), or a pharmaceutically acceptable salt, solvate or prodrug thereof, for the preparation of a pharmaceutical composition for treating a condition benefiting from a reduction of reactive oxygen species in a patient in need thereof.

30

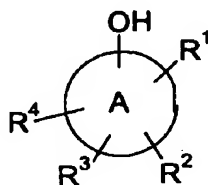
67. Use according to claim 65 or claim 66, wherein the condition to be treated is the aging process, damage of heart tissue, damage of neuronal tissue, Alzheimer's disease, cancer, or cataract.

35

68. Use according to any of claims 1 to 67, wherein the compound is a chemical uncoupler as defined in Assay (II), as described in the specification.

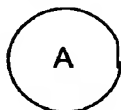
5 69. Use according to any of claims 1 to 68, wherein the compound is a cation.

70. Use according to any of claims 1 to 68, wherein the compound is of the general formula (I)



(I)

wherein



is an aryl, or heteroaryl,

15 R^1 is halogen, $-\text{CHO}$, $-\text{CO}_2R^{32}$, $-\text{COR}^{32}$, $-\text{SO}_3\text{H}$, $-\text{CCl}_3$, $-\text{CF}_3$, $-\text{NO}$, $-\text{NO}_2$, $-\text{CN}$, $-\text{CH}=\text{CH}-R^{33}$, $-\text{C}(R^{33})(R^{34})$, $-\text{SOR}^{32}$, $-\text{SO}_2R^{32}$ or aryl substituted with from one to five substituents selected from halogen, $-\text{CHO}$, $-\text{CO}_2R^{32}$, $-\text{COR}^{32}$, $-\text{SO}_3\text{H}$, $-\text{CCl}_3$, $-\text{CF}_3$, $-\text{NO}$, $-\text{NO}_2$, $-\text{CN}$, $-\text{CH}=\text{CH}-R^{33}$, $-\text{CH}(R^{33})(R^{34})$, $-\text{SOR}^{32}$, $-\text{SO}_2R^{32}$, wherein

R^{32} is hydrogen, alkyl, aryl, or heteroaryl; and

20 R^{33} and R^{34} independently of each other are halogen, $-\text{CHO}$, $-\text{CO}_2R^{35}$, $-\text{COR}^{35}$, $-\text{SO}_3\text{H}$, $-\text{CCl}_3$, $-\text{CF}_3$, $-\text{NO}$, $-\text{NO}_2$, $-\text{CN}$, $-\text{SOR}^{35}$, $-\text{SO}_2R^{35}$, wherein

R^{35} is hydrogen or alkyl;

and is attached on a carbon atom adjacent to the carbon atom to which the hydroxy group is attached;

R^2 is $\text{C}(\text{X})_3$, NO_2 , alkyl, nitro, halogen, alkyl-O-, alkyl-C(O)-, alkyl-C(O)-O-, or aryl, wherein

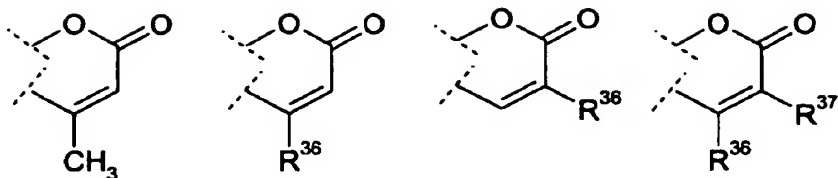
25 X is halogen; and

R^3 and R^4 independently of each other are hydrogen, alkyl, nitro, halogen, alkyl-O-, alkyl-C(O)-, alkyl-C(O)-O-, or aryl;

or

R^2 and R^3 together forms one of the diradicals

79



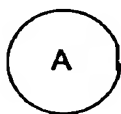
wherein

R^{36} and R^{37} , independently of each other, are hydrogen, halogen, $C(X)_3$, nitro, cyano, alkyl, alkyl-O-, alkyl-C(O)-, or aryl, wherein

5 X is halogen;

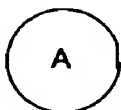
and where the two connecting atoms are connected to adjacent carbon atoms; and R^4 is hydrogen, halogen, $C(X)_3$, nitro, cyano, alkyl, alkyl-O-, alkyl-C(O)-, or aryl, or a pharmaceutically acceptable salt, solvate or prodrug thereof.

10 71. Use according to claim 70, wherein

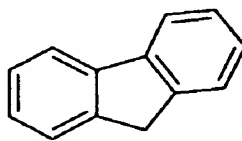
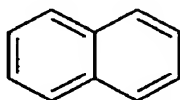
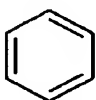


is an aryl.

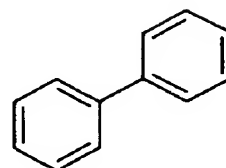
72. Use according to claim 71, wherein



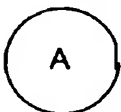
15 is an aryl selected from

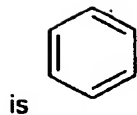


, or

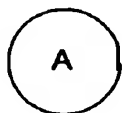


73. Use according to claim 72, wherein





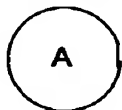
74. Use according to claim 70, wherein



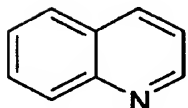
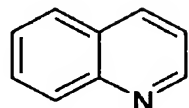
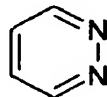
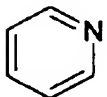
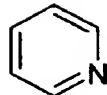
is a heteroaryl.

5

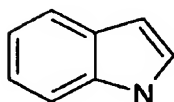
75. Use according to claim 74, wherein



is a heteroaryl selected from



, or



10

76. Use according to any of claims 70 to 75, wherein

R^1 is halogen, $-\text{CHO}$, $-\text{CO}_2R^{32}$, $-\text{COR}^{32}$, $-\text{SO}_3\text{H}$, $-\text{CCl}_3$, $-\text{CF}_3$, $-\text{NO}$, $-\text{NO}_2$, $-\text{CN}$,

$-\text{CH}=\text{CH}-R^{33}$, $-\text{CH}(R^{33})(R^{34})$, $-\text{SOR}^{32}$, $-\text{SO}_2R^{32}$, wherein

R^{32} , R^{33} and R^{34} are as defined in claim 70.

15

77. Use according to any of claims 70 to 76, wherein R^{32} is hydrogen.

78. Use according to any of claims 70 to 77, wherein R^{33} and R^{34} independently of each other are halogen, $-\text{CHO}$, $-\text{CO}_2\text{H}$, $-\text{COH}$, $-\text{SO}_3\text{H}$, $-\text{CCl}_3$, $-\text{CF}_3$, $-\text{NO}$, $-\text{NO}_2$, $-\text{CN}$, $-\text{SOH}$, $-\text{SO}_2\text{H}$.

79. Use according to any of claims 70 to 75, wherein R^1 is nitro.

80. Use according to any of claims 70 to 79, wherein R^2 is $C(X)_3$, alkyl, nitro, halogen, alkyl-O-, or alkyl-C(O)-, wherein X is halogen.

81. Use according to claim 80, wherein R^2 is alkyl.

82. Use according to claim 81, wherein R^2 is C_{1-6} -alkyl.

83. Use according to claim 82, wherein R^2 is selected from methyl, ethyl, propyl, isopropyl, butyl, tert-butyl, pentyl and hexyl.

84. Use according to claim 83, wherein R^2 is methyl.

85. Use according to claim 80, wherein R^2 is alkyl-O-.

86. Use according to claim 85, wherein R^2 is C_{1-6} -alkyl-O-.

87. Use according to claim 86, wherein R^2 is C_{1-6} -alkyl-O-, wherein the alkyl is selected from methyl, ethyl, propyl, isopropyl, butyl, tert-butyl, pentyl and hexyl.

88. Use according to claim 87, wherein R^2 is CH_3O -.

89. Use according to claim 80, wherein R^2 is alkyl-C(O)-.

90. Use according to claim 89, wherein R^2 is C_{1-6} -alkyl-C(O)-.

91. Use according to claim 90, wherein R^2 is C_{1-6} -alkyl-C(O)-, wherein the alkyl is selected from methyl, ethyl, propyl, isopropyl, butyl, tert-butyl, pentyl and hexyl.

92. Use according to claim 91, wherein R^2 is $CH_3-C(O)$ -.

93. Use according to claim 80, wherein R^2 is alkyl-C(O)-O-.

94. Use according to claim 93, wherein R^2 is C_{1-6} -alkyl-C(O)-O-.

95. Use according to claim 94, wherein R^2 is C_{1-6} -alkyl-C(O)-O-, wherein the alkyl is selected from methyl, ethyl, propyl, isopropyl, butyl, tert-butyl, pentyl and hexyl.

5

96. Use according to claim 95, wherein R^2 is CH_3 -C(O)-O-.

97. Use according to claim 80, wherein R^2 is $C(X)_3$, wherein X is halogen.

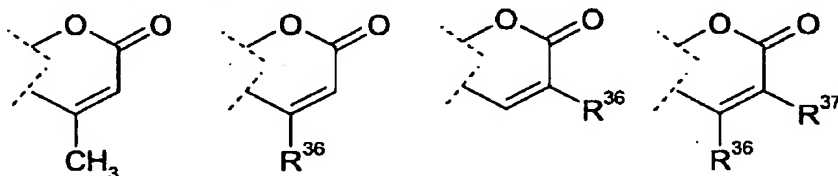
10 98. Use according to claim 97, wherein X is chloro.

99. Use according to claim 97, wherein X is fluoro.

100. Use according to any of claims 70 to 99, wherein R^3 is hydrogen.

15

101. Use according to any of claims 70 to 79, wherein R^2 and R^3 together forms the diradical



wherein R^{36} and R^{37} , independently of each other, are hydrogen, halogen, $C(X)_3$, nitro, cyano, alkyl, alkyl-O-, alkyl-C(O)-, or aryl, wherein

20 X is halogen.

102. Use according to claim 101, wherein R^{36} is hydrogen.

103. Use according to claim 101 or claim 102, wherein R^{37} is hydrogen.

25

104. Use according to any of claims 70 to 103, wherein R^4 is hydrogen.

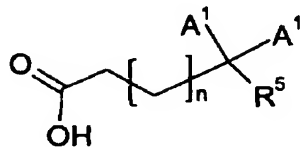
105. Use according to claim 70, wherein the compound is

4-methoxy-2-nitrophenol,

30 4-hydroxy-3-nitroacetophenone, or

7-hydroxy-4-methyl-8-nitro-chromen-2-one.

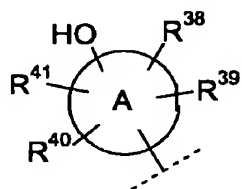
106. Use according to any of claims 1 to 68, where the compound is of the general formula (II)



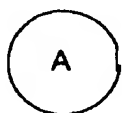
(II)

5

wherein A¹ is



, wherein



is an aryl, or heteroaryl,

10

R³⁸ is halogen, -CHO, -CO₂R⁴², -COR⁴², -SO₃H, -CCl₃, -CF₃, -NO, -NO₂, -CN, -SOR⁴², or -SO₂R⁴², wherein

R⁴² is hydrogen or alkyl;

and is attached to a carbon atom adjacent to the carbon atom to which the hydroxy group is attached;

15

R³⁹, R⁴⁰, and R⁴¹ independently of each other are hydrogen, alkyl, nitro, cyano, halogen, alkyl-O-, alkyl-C(O)-, alkyl-C(O)-O-, or aryl;

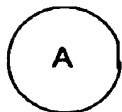
R⁵ is hydrogen or alkyl; and

n is an integer of from 0 to 10

or a pharmaceutically acceptable salt, solvate or prodrug thereof.

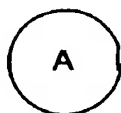
20

107. Use according to claim 106, wherein

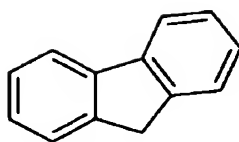
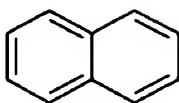
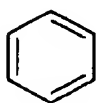


is an aryl.

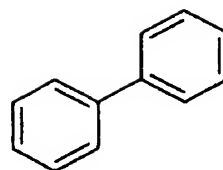
108. Use according to claim 107, wherein



is an aryl selected from

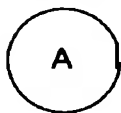


, or



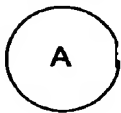
5

109. Use according to claim 108, wherein



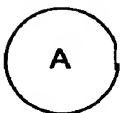
is

10 110. Use according to claim 106, wherein



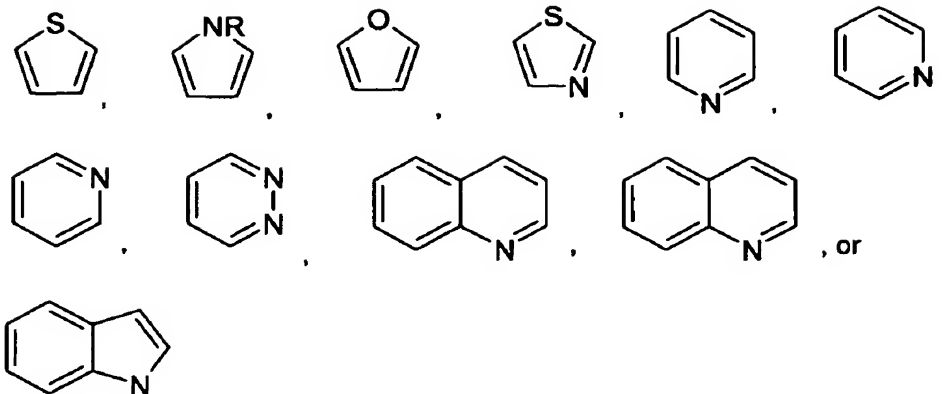
is a heteroaryl.

111. Use according to claim 74. wherein



15 is a heteroaryl selected from

85



112. Use according to any of claims 106 to 111, wherein

R³⁸ is halogen, -CHO, -CO₂H, -COH, -SO₃H, -CCl₃, -CF₃, -NO, -NO₂, -CN, -SOH, or -SO₂H.

113. Use according to claim 112, wherein R³⁸ is nitro.

114. Use according to any of claims 106 to 113, wherein at least one of R³⁹, R⁴⁰, and R⁴¹ is hydrogen.

115. Use according to claim 114, wherein at least two of R³⁹, R⁴⁰, and R⁴¹ is hydrogen.

116. Use according to claim 115, wherein R³⁹, R⁴⁰, and R⁴¹ are hydrogen.

117. Use according to any of claims 106 to 116, wherein n is an integer of from 0 to 6.

118. Use according to claim 117, wherein n is an integer of from 0 to 2.

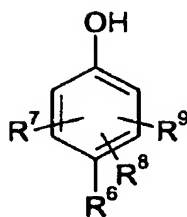
119. Use according to claim 118, wherein n is an integer of 1 or 2.

120. Use according to claim 119, wherein n is an integer of 1.

121. Use according to claim 119, wherein the compound is

4,4-bis-(4-hydroxy-3-nitrophenyl)-valeric acid.

122. Use according to any of claims 1 to 68, where the compound is of the general formula (III)



(III)

5 wherein

R^6 is halogen, $-\text{CHO}$, $-\text{CO}_2R^{43}$, $-\text{COR}^{43}$, $-\text{SO}_3\text{H}$, $-\text{CCl}_3$, $-\text{CF}_3$, $-\text{NO}$, $-\text{NO}_2$, $-\text{CN}$, $-\text{CH}=\text{CH}-R^{44}$, $-\text{C}(R^{44})(R^{45})$, $-\text{SOR}^{43}$, $-\text{SO}_2R^{43}$ or aryl substituted with from one to five substituents selected from halogen, $-\text{CHO}$, $-\text{CO}_2R^{43}$, $-\text{COR}^{43}$, $-\text{SO}_3\text{H}$, $-\text{CCl}_3$, $-\text{CF}_3$, $-\text{NO}$, $-\text{NO}_2$, $-\text{CN}$, $-\text{CH}=\text{CH}-R^{44}$, $-\text{CH}(R^{44})(R^{45})$, $-\text{SOR}^{43}$, $-\text{SO}_2R^{43}$, wherein

10 R^{43} is hydrogen or alkyl; and

R^{44} and R^{45} independently of each other are halogen, $-\text{CHO}$, $-\text{CO}_2R^{48}$, $-\text{COR}^{46}$, $-\text{SO}_3\text{H}$, $-\text{CCl}_3$, $-\text{CF}_3$, $-\text{NO}$, $-\text{NO}_2$, $-\text{CN}$, $-\text{SOR}^{48}$, $-\text{SO}_2R^{48}$, wherein

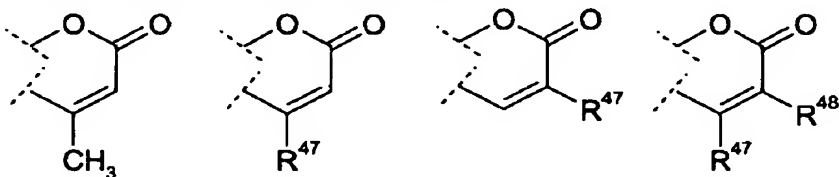
R^{48} is hydrogen, alkyl, or aryl;

R^7 is alkyl, nitro, halogen, alkyl-O-, alkyl-C(O)-, or alkyl-C(O)-O-; and

15 R^8 and R^9 independently of each other are hydrogen, alkyl, nitro, halogen, alkyl-O-, alkyl-C(O)-, alkyl-C(O)-O-, or aryl;

or

R^7 and R^8 together forms the diradical



20 wherein R^{47} and R^{48} , independently of each other, are hydrogen, alkyl, nitro, halogen, alkyl-O-, alkyl-C(O)-, or alkyl-C(O)-O-,

where the two valence atoms are connected to adjacent carbon atoms; and

R^9 is hydrogen, alkyl, nitro, halogen, alkyl-O-, or alkyl-C(O)-

or a pharmaceutically acceptable salt, solvate or prodrug thereof.

25

123. Use according to claim 122, wherein

R^6 is halogen, $-\text{CHO}$, $-\text{CO}_2R^{43}$, $-\text{COR}^{43}$, $-\text{SO}_3\text{H}$, $-\text{CCl}_3$, $-\text{CF}_3$, $-\text{NO}$, $-\text{NO}_2$, $-\text{CN}$, $-\text{CH}=\text{CH}-R^{44}$, $-\text{CH}(R^{44})(R^{45})$, $-\text{SOR}^{43}$, $-\text{SO}_2R^{43}$, wherein R^{43} , R^{44} and R^{45} are as defined in claim 122.

- 5 124. Use according to claim 122 or claim 123, wherein R^{43} is hydrogen.
125. Use according to any of claims 122 to 124, wherein R^{44} and R^{45} independently of each other are halogen, $-\text{CHO}$, $-\text{CO}_2\text{H}$, $-\text{COH}$, $-\text{SO}_3\text{H}$, $-\text{CCl}_3$, $-\text{CF}_3$, $-\text{NO}$, $-\text{NO}_2$, $-\text{CN}$, $-\text{SOH}$, $-\text{SO}_2\text{H}$.
- 10 126. Use according to claim 123, wherein R^6 is cyano or nitro
127. Use according to claim 126, wherein R^6 is nitro.
128. Use according to any of claims 122 to 127, wherein R^7 is alkyl, nitro, halogen, alkyl-O-,
15 or alkyl-C(O)-.
129. Use according to claim 128, wherein R^7 is alkyl.
130. Use according to claim 129, wherein R^7 is C_{1-6} -alkyl.
- 20 131. Use according to claim 130, wherein R^7 is selected from methyl, ethyl, propyl, isopropyl, butyl, tert-butyl, pentyl, hexyl, or 1,1-dimethylpropyl.
132. Use according to claim 131, wherein R^7 is methyl.
- 25 133. Use according to claim 128, wherein R^7 is alkyl-O-.
134. Use according to claim 133, wherein R^7 is C_{1-6} -alkyl-O-.
- 30 135. Use according to claim 134, wherein R^7 is C_{1-6} -alkyl-O-, wherein the alkyl is selected from methyl, ethyl, propyl, isopropyl, butyl, tert-butyl, pentyl and hexyl.
136. Use according to claim 135, wherein R^7 is CH_3O -.
- 35 137. Use according to claim 128, wherein R^7 is alkyl-C(O)-.

138. Use according to claim 137, wherein R^7 is C_{1-6} -alkyl-C(O)-.

139. Use according to claim 138, wherein R^7 is C_{1-6} -alkyl-C(O)-, wherein the alkyl is selected from methyl, ethyl, propyl, isopropyl, butyl, tert-butyl, pentyl and hexyl.

140. Use according to claim 139, wherein R^7 is CH_3 -C(O)-.

141. Use according to claim 128, wherein R^7 is alkyl-C(O)-O-.

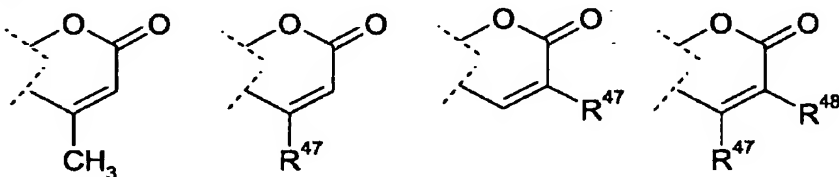
142. Use according to claim 141, wherein R^7 is C_{1-6} -alkyl-C(O)-O-.

143. Use according to claim 142, wherein R^7 is C_{1-6} -alkyl-C(O)-O-, wherein the alkyl is selected from methyl, ethyl, propyl, isopropyl, butyl, tert-butyl, pentyl and hexyl.

144. Use according to claim 143, wherein R^7 is CH_3 -C(O)-O-.

145. Use according to any of claims 122 to 144, wherein R^8 is hydrogen.

146. Use according to any of claims 122 to 127, wherein R^7 and R^8 together forms the diradical



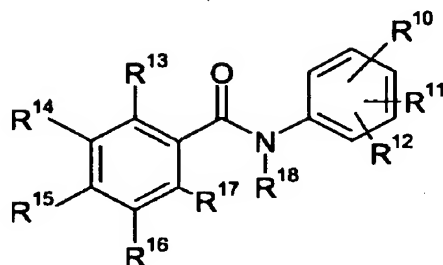
wherein R^{47} and R^{48} , independently of each other, are hydrogen, alkyl, nitro, halogen, alkyl-O-, alkyl-C(O)-, or alkyl-C(O)-O-.

147. Use according to claim 146, wherein R^{47} is hydrogen.

148. Use according to claim 146 or claim 147, wherein R^{48} is hydrogen.

149. Use according to claim 148, wherein R^9 is hydrogen.

150. Use according to any of claims 1 to 68, where the compound is of the general formula (IV)



(IV)

5 wherein

R¹⁰, R¹¹ and R¹² independently of each other are hydrogen, trifluoromethyl, nitro, cyano, alkyl-S-, SO_y, R⁴⁹-O-, N(R⁵⁰)(R⁵¹)-, alkyl, halogen, or aryl-S-, wherein y is an integer of 1 or 2;

R⁴⁹, R⁵⁰ and R⁵¹ independently of each other are hydrogen or alkyl;

10 wherein at least one of R¹⁰, R¹¹ and R¹² is different from hydrogen;

and

R¹³, R¹⁴, R¹⁵, R¹⁶ and R¹⁷ independently of each other are hydrogen, halogen, hydroxy, halogen, cyano, or alkyl, aryl, aryl-S-, or heteroaryl, optionally substituted with halogen;

15

or

R¹³ and R¹⁴ together form a conjugated alkenylene, which together with the benzene ring forms a fused aromatic ring system, which may optionally be substituted with one or more substituents selected from the group consisting of hydroxy, methyl, halogen, CF₃, alkyl-O-, nitro, and cyano;

and

R¹⁵, R¹⁶ and R¹⁷, independently of each other, are hydrogen, halogen, hydroxy, halogen, or alkyl optionally substituted with halogen

20

or

R¹⁴ and R¹⁵ together form a conjugated alkenylene, which together with the benzene ring forms a fused aromatic ring system, which may optionally be substituted with one or more substituents selected from the group consisting of hydroxy, methyl, halogen, CF₃, alkyl-O-, nitro, and cyano;

and

25

R^{13} , R^{18} and R^{17} independently of each other are hydrogen, halogen, hydroxy, halogen, or alkyl, aryl or heteroaryl, optionally substituted with halogen;

and

5 R^{18} is hydrogen;

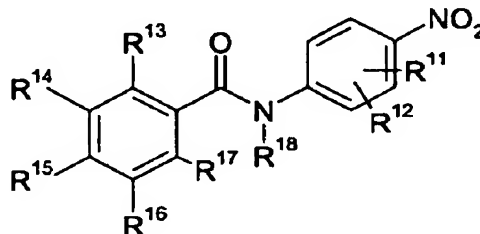
or a pharmaceutically acceptable salt, solvate or prodrug thereof.

151. Use according to claim 150, wherein at least two of R^{10} , R^{11} and R^{12} is different from hydrogen.

10

152. Use according to claim 150 or claim 151, wherein R^{10} is nitro.

153. Use according to claim 152, wherein the compound is of the general formula (IVa)



15

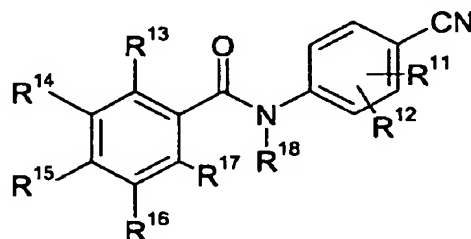
(IVa)

wherein R^{11} , R^{12} , R^{13} , R^{14} , R^{15} , R^{16} , R^{17} , and R^{18} is as defined in claim 150.

154. Use according to claim 150 or claim 151, wherein R^{10} is cyano.

20

155. Use according to claim 154, wherein the compound is of the general formula (IVb)



(IVb)

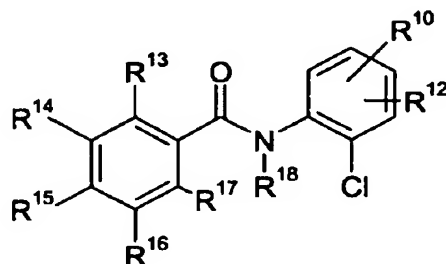
wherein R^{11} , R^{12} , R^{13} , R^{14} , R^{15} , R^{16} , R^{17} , and R^{18} is as defined in claim 150.

25

156. Use according to any of claims 150 to 155, wherein R^{11} is halogen.

157. Use according to claim 156, wherein R^{11} is chloro.

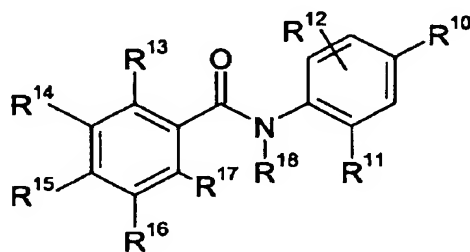
158. Use according to claim 150, wherein the compound is of the general formula (IVc)



(IVc)

wherein R^{11} , R^{12} , R^{13} , R^{14} , R^{15} , R^{16} , R^{17} , and R^{18} is as defined in claim 150.

159. Use according to claim 150 or claim 151, wherein the compound is of the general formula (IVd)



(IVd)

wherein R^{11} , R^{12} , R^{13} , R^{14} , R^{15} , R^{16} , R^{17} , and R^{18} is as defined in claim 150, with the proviso that R^{10} and R^{11} are different from hydrogen.

160. Use according to claim 159, wherein R^{10} is nitro or cyano.

161. Use according to any of claims 159 to 160, wherein R^{11} is halogen.

162. Use according to claim 161, wherein R^{11} is chloro.

163. Use according to any of claims 150 to 162, wherein R^{12} is hydrogen.

164. Use according to any of claims 150 to 163, wherein at least one of R^{13} , R^{14} , R^{15} , R^{16} and R^{17} is different from hydrogen.

5 165. Use according to claim 164, wherein at least two of R^{13} , R^{14} , R^{15} , R^{16} and R^{17} are different from hydrogen

166. Use according to claim 164 or 165, wherein at least one of R^{13} , R^{14} , R^{15} , R^{16} and R^{17} is hydroxy.

10 167. Use according to any of claims 164 to 166, wherein at least one of R^{13} , R^{14} , R^{15} , R^{16} and R^{17} is alkyl.

168. Use according to claim 167, wherein at least one of R^{13} , R^{14} , R^{15} , R^{16} and R^{17} is C_{1-6} -alkyl.

15 169. Use according to claim 168, wherein at least one of R^{13} , R^{14} , R^{15} , R^{16} and R^{17} is neopentyl, adamantyl, tert-butyl, 1-methylcyclopentyl, cyclopropyl, cyclobutyl, isopropyl, or 1,1-dimethylpropyl.

20 170. Use according to any of claims 164 to 169, wherein at least one of R^{13} , R^{14} , R^{15} , R^{16} and R^{17} is methyl.

171. Use according to any of claims 164 to 170, wherein at least one of R^{13} , R^{14} , R^{15} , R^{16} and R^{17} is alkyl substituted with halogen.

25 172. Use according to claim 171, wherein at least one of R^{13} , R^{14} , R^{15} , R^{16} and R^{17} is trifluoromethyl.

30 173. Use according to claim 172, wherein at least two of R^{13} , R^{14} , R^{15} , R^{16} and R^{17} is trifluoromethyl.

174. Use according to any of claims 164 to 173, wherein R^{17} is alkyl.

35 175. Use according to any of claims 164 to 173, wherein R^{17} is C_{1-6} -alkyl.

176. Use according to claim 175, wherein R¹⁷ is neopentyl, adamantyl, tert-butyl, isopropyl, or 1,1-dimethylpropyl.

177. Use according to claim 176, wherein R¹⁷ is isopropyl.

5

178. Use according to claim 176, wherein R¹⁷ is tert-butyl.

179. Use according to claim 175, wherein R¹⁷ is methyl.

10

180. Use according to any of claims 164 to 179, wherein R¹³ is hydroxy.

181. Use according to any of claims 164 to 180, wherein R¹⁴ is alkyl.

182. Use according to claim 181, wherein R¹⁴ is C₁₋₆-alkyl.

15

183. Use according to claim 182, wherein R¹⁴ is neopentyl, adamantyl, tert-butyl, isopropyl, or 1,1-dimethylpropyl.

184. Use according to claim 183, wherein R¹⁴ is isopropyl.

20

185. Use according to claim 183, wherein R¹⁴ is tert-butyl.

186. Use according to claim 182, wherein R¹⁴ is methyl.

25

187. Use according to any of claims 164 to 180, wherein R¹⁴ is aryl-S-.

188. Use according to claim 187, wherein R¹⁴ is phenyl-S-.

189. Use according to any of claims 164 to 180, wherein R¹⁴ is trifluoromethyl.

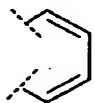
30

190. Use according to 189, wherein R¹⁴ and R¹⁶ are trifluoromethyl.

191. Use according to claim 165, wherein R¹³ and R¹⁴ together form a conjugated C₃₋₅-alkenylene, which together with the benzene ring forms a fused aromatic ring system,

which may optionally be substituted with one or more substituents selected from the group consisting of hydroxy, methyl, halogen, CF_3 , alkyl-O-, nitro, and cyano.

192. Use according to claim 191, wherein R^{13} and R^{14} together form



5, which may optionally be substituted with one or more substituents selected from the group consisting of hydroxy, methyl, halogen, CF_3 , alkyl-O-, nitro, and cyano.

193. Use according to claim 191 or 192, wherein R^{15} , R^{16} , and R^{17} all are hydrogen.

10 194. Use according to claim 191 or 192, wherein at least one of R^{15} , R^{16} , and R^{17} is different from hydrogen.

195. Use according to claim 194, wherein at least two of R^{15} , R^{16} , and R^{17} is different from hydrogen.

15

196. Use according to claim 194 or 195, wherein at least one of R^{15} , R^{16} , and R^{17} is hydroxy.

197. Use according to any of claims 194 to 196, wherein at least one of R^{15} , R^{16} , and R^{17} is alkyl, aryl or heteroaryl, optionally substituted with halogen.

20

198. Use according to claim 197, wherein at least one of R^{15} , R^{16} , and R^{17} is C_{1-6} -alkyl, aryl or heteroaryl, optionally substituted with halogen.

25

199. Use according to claim 198, wherein at least one of R^{15} , R^{16} , and R^{17} is neopentyl, adamantyl, tert-butyl, isopropyl, or 1,1-dimethylpropyl.

200. Use according to claim 198, wherein at least one of R^{15} , R^{16} , and R^{17} is C_{1-6} -alkyl substituted with halogen.

30 201. Use according to claim 200, wherein at least one of R^{15} , R^{16} , and R^{17} is trifluoromethyl.

202. Use according to claim 201, wherein at least two of R^{15} , R^{16} , and R^{17} is trifluoromethyl.

203. Use according to any of claims 194 to 201, wherein at least one of R^{15} , R^{16} , and R^{17} is methyl.

204. Use according to any of claims 194 to 203, wherein R^{17} is alkyl.

5

205. Use according to claim 204, wherein R^{17} is C_{1-6} -alkyl.

206. Use according to claim 205, wherein R^{17} is neopentyl, adamantyl, tert-butyl, isopropyl, or 1,1-dimethylpropyl.

10

207. Use according to claim 206, wherein R^{17} is isopropyl.

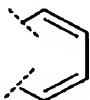
208. Use according to claim 206, wherein R^{17} is tert-butyl.

15 209. Use according to claim 205, wherein R^{17} is methyl.

210. Use according to claim 165, wherein R^{14} and R^{15} together form a conjugated C_{3-5} -alkenylene, which together with the benzene ring forms a fused aromatic ring system, which may optionally be substituted with one or more substituents selected from the group consisting of hydroxy, methyl, halogen, CF_3 , alkyl-O-, nitro, and cyano.

20

211. Use according to claim 210, wherein R^{14} and R^{15} together form



, which may optionally be substituted with one or more substituents selected from the group consisting of hydroxy, methyl, halogen, CF_3 , alkyl-O-, nitro, and cyano.

25

212. Use according to claim 210 or 211, wherein R^{13} , R^{16} , and R^{17} all are hydrogen.

213. Use according to claim 210 or 211, wherein at least one of R^{13} , R^{16} , and R^{17} is different from hydrogen.

30

214. Use according to claim 213, wherein at least two of R^{13} , R^{16} , and R^{17} is different from hydrogen.

215. Use according to claim 213 or 214, wherein at least one of R^{13} , R^{16} , and R^{17} is hydroxy.

216. Use according to any of claims 213 to 215, wherein at least one of R^{13} , R^{16} , and R^{17} is alkyl, aryl or heteroaryl, optionally substituted with halogen.

5

217. Use according to claim 216, wherein at least one of R^{13} , R^{16} , and R^{17} is C_{1-6} -alkyl, aryl or heteroaryl, optionally substituted with halogen.

10

218. Use according to claim 217, wherein at least one of R^{13} , R^{16} , and R^{17} is neopentyl, adamantyl, tert-butyl, isopropyl, or 1,1-dimethylpropyl.

219. Use according to claim 217, wherein at least one of R^{13} , R^{16} , and R^{17} is C_{1-6} -alkyl substituted with halogen.

15

220. Use according to claim 219, wherein at least one of R^{13} , R^{16} , and R^{17} is trifluoromethyl.

221. Use according to claim 220, wherein at least two of R^{13} , R^{16} , and R^{17} is trifluoromethyl.

20

222. Use according to any of claims 213 to 220, wherein at least one of R^{13} , R^{16} , and R^{17} is methyl.

223. Use according to any of claims 213 to 222, wherein R^{17} is alkyl.

224. Use according to claim 223, wherein R^{17} is C_{1-6} -alkyl.

25

225. Use according to claim 224, wherein R^{17} is neopentyl, adamantyl, tert-butyl, isopropyl, or 1,1-dimethylpropyl.

226. Use according to claim 225, wherein R^{17} is isopropyl.

30

227. Use according to claim 225, wherein R^{17} is tert-butyl.

228. Use according to claim 224, wherein R^{17} is methyl.

35

229. Use according to any of claims 213 to 228, wherein R^{13} is hydroxy.

230. Use according to any of claims 150 to 163, wherein R^{13} , R^{14} , R^{15} , R^{16} and R^{17} are all hydrogen.

5 231. Use according to any of claims 150 to 230, wherein R^{18} is hydrogen.

232. Use according to any of claims 150 to 230, wherein R^{18} is alkyl.

233. Use according to claim 232, wherein R^{18} is C_{1-6} -alkyl.

10

234. Use according to claim 233, wherein R^{18} is selected from methyl, ethyl, propyl, isopropyl, butyl, tert-butyl, pentyl and hexyl.

235. Use according to claim 234, wherein R^{18} is methyl.

15

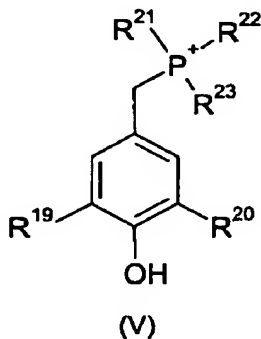
236. Use according to claim 150, wherein the compound is
tert-butyl-5-chloro-N-(2-chloro-4-nitrophenyl)-2-hydroxy-6-methylbenzamide,
N-1-[4-cyano-3-(trifluoromethyl)phenyl]-3,5-di(trifluoromethyl)benzamide,
N-(4-cyanophenyl)benzamide,

20

2'-chloro-1-hydroxy-4'-nitro-2-naphthanilide,
N-(2-chloro-4-bromophenyl)-5-bromosalicylanilide,
N-(2-chloro-4-nitrophenyl)-3-tert-butyl-6-methylsalicylanilide,
3,6-dinitrocarbazole, or
N-(3-cyano-4-phenylsulfanyl-phenyl)-3-trifluoromethyl-benzamide.

25

237. Use according to any of claims 1 to 68, where the compound is of the general formula (V)



wherein R^{19} and R^{20} independently of each other are alkyl;

and

R^{21} , R^{22} and R^{23} independently of each other are selected from alkyl, cycloalkyl, or aryl or a pharmaceutically acceptable salt, solvate or prodrug thereof.

5

238. Use according to claim 237, wherein R^{19} and R^{20} independently of each other are C_{1-6} -alkyl.

10

239. Use according to claim 238, wherein R^{19} and R^{20} independently of each other are neopentyl, adamantyl, tert-butyl, isopropyl, or 1,1-dimethylpropyl.

240. Use according to claim 239, wherein R^{19} and R^{20} independently of each other are isopropyl.

15

241. Use according to claim 239, wherein R^{19} and R^{20} independently of each other are tert-butyl.

242. Use according to any of claims 237 to 241, wherein R^{21} , R^{22} and R^{23} independently of each other are C_{1-12} -alkyl, C_{3-8} -cycloalkyl, or aryl.

20

243. Use according to claim 242, wherein R^{21} , R^{22} and R^{23} independently of each other are aryl.

244. Use according to claim 243, wherein at least one of R^{21} , R^{22} and R^{23} is phenyl.

25

245. Use according to claim 244, wherein R^{21} , R^{22} and R^{23} are phenyl.

246. Use according to any of claims 237 to 241, wherein R^{21} , R^{22} and R^{23} independently of each other are cycloalkyl.

30

247. Use according to claim 242 or 246, wherein R^{21} , R^{22} and R^{23} independently of each other are C_{3-8} -cycloalkyl.

248. Use according to claim 247, wherein at least one of R^{21} , R^{22} and R^{23} is cyclohexyl.

35

249. Use according to claim 248, wherein R^{21} , R^{22} and R^{23} are cyclohexyl.

250. Use according to any of claims 237 to 241, wherein R^{21} , R^{22} and R^{23} independently of each other are alkyl.

5

251. Use according to claim 242 or 250, wherein R^{21} , R^{22} and R^{23} independently of each other are C_{1-12} -alkyl.

10

252. Use according to claim 251, wherein R^{21} , R^{22} and R^{23} independently of each other are C_{1-8} -alkyl.

253. Use according to claim 252, wherein R^{21} , R^{22} and R^{23} independently of each other are C_{1-8} -alkyl.

15

254. Use according to claim 252, wherein at least one of R^{21} , R^{22} and R^{23} is octyl.

255. Use according to claim 254, wherein R^{21} , R^{22} and R^{23} are octyl.

20

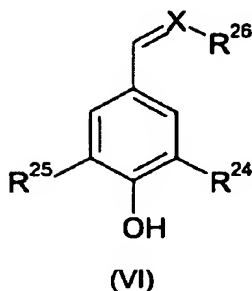
256. Use according to claim 252 or claim 253, wherein at least one of R^{21} , R^{22} and R^{23} is heptyl.

257. Use according to claim 256, wherein R^{21} , R^{22} and R^{23} are heptyl.

25

258. Use according to claim 237, wherein the compound is
(3,5-di-tert-butyl-4-hydroxybenzyl)triphenylphosphonium bromide,
(3,5-di-tert-butyl-4-hydroxybenzyl)tricyclohexylphosphonium bromide,
(3,5-di-tert-butyl-4-hydroxybenzyl)tributylphosphonium bromide, or
(3,5-di-tert-butyl-4-hydroxybenzyl)trioctylphosphonium bromide.

259. Use according to any of claims 1 to 68, where the compound is of the general formula (VI)



5 wherein

R^{24} and R^{25} independently of each other are alkyl or cycloalkyl;
and

X is $=C(R^{52})-$; wherein

R^{52} is hydrogen, cyano, nitro, $R^{53}-S(O)_2-$, tetrazole, alkyl-S-, alkyl-C(O)-, or alkyl-O-C(O)-, wherein

R^{53} is hydrogen, or alkyl or phenyl optionally substituted with halogen; and

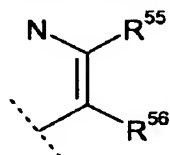
R^{26} is cyano, nitro, $R^{54}-S(O)_2-$, tetrazole, alkyl-C(O)-, or alkyl-O-C(O)-, wherein

R^{54} is hydrogen, or alkyl or phenyl optionally substituted with halogen;

15 or

X is $=N-$, and

R^{26} is cyano, nitro, $R^{54}-S(O)_2-$, alkyl-C(O)-, alkyl-O-C(O)-, or



wherein

R^{54} is hydrogen, or alkyl or phenyl optionally substituted with halogen; and

R^{55} and R^{56} independently of each other are cyano, nitro, $R^{57}-S(O)_2-$, alkyl-C(O)-, or alkyl-O-C(O)-, wherein

R^{57} is hydrogen, or alkyl or phenyl optionally substituted with halogen;

25 or a pharmaceutically acceptable salt, solvate or prodrug thereof.

260. Use according to claim 259, wherein R^{24} and R^{25} independently of each other are alkyl.

261. Use according to claim 260, wherein R^{24} and R^{25} independently of each other are C_{1-6} -alkyl.

5 262. Use according to claim 261, wherein R^{24} and R^{25} independently of each other are neopentyl, adamantyl, tert-butyl, isopropyl, or 1,1-dimethylpropyl.

263. Use according to claim 262, wherein R^{24} and R^{25} are tert-butyl.

10 264. Use according to claim 262, wherein R^{24} and R^{25} are isopropyl.

265. Use according to claim 259, wherein R^{24} and R^{25} independently of each other are cycloalkyl.

15 266. Use according to claim 265, wherein R^{24} and R^{25} independently of each other are C_{3-6} -cycloalkyl.

267. Use according to claim 259, wherein the compound is

2-cyano-3-(3,5-di-tert-butyl-4-hydroxyphenyl)-acrylic acid ethyl ester,

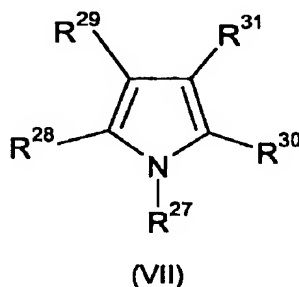
20 2-(3,5-di-tert-butyl-4-hydroxy-benzylidene)-malonic acid diethyl ester,

2-amino-S-[(3,5-di-tert-butyl-4-hydroxybenzylidene)-amino]-but-2-enedinitrile, or

2-(3,5-di-tert-butyl-4-hydroxy-benzylidene)-indan-1,3-dione.

268. Use according to any of claims 1 to 68, where the compound is of the general formula (VII)

25



wherein

R^{27} is hydrogen or alkyl-O-CH₂;

R²⁸ and R²⁹ independently of each other are hydrogen, halogen, dicyanovinyl, cyano, nitro, dinitrovinyl, alkyl optionally substituted with halogen, or aryl optionally substituted with one or more substituents selected from the group consisting of halogen, dicyanovinyl, cyano, nitro, and dinitrovinyl,

or

R²⁸ and R²⁹ together forms a benzene ring optionally substituted with one or more substituents selected from the group consisting of halogen, C₁₋₆-alkyl, dicyanovinyl, cyano, nitro, and dinitrovinyl;

and

R³⁰ is halogen, dicyanovinyl, cyano, nitro, dinitrovinyl, alkyl optionally substituted with halogen, or aryl optionally substituted with one or more substituents selected from the group consisting of halogen, dicyanovinyl, cyano, nitro, and dinitrovinyl; and R³¹ is hydrogen, halogen, dicyanovinyl, cyano, nitro, dinitrovinyl, alkyl optionally substituted with halogen, or aryl optionally substituted with one or more substituents selected from the group consisting of halogen, dicyanovinyl, cyano, nitro, and dinitrovinyl;

or

R³⁰ and R³¹ together forms a benzene ring optionally substituted with one or more substituents selected from the group consisting of halogen, C₁₋₆-alkyl, dicyanovinyl, cyano, nitro, and dinitrovinyl,

or a pharmaceutically acceptable salt, solvate or prodrug thereof.

269. Use according to claim 268, wherein R²⁷ is hydrogen or C₁₋₆-alkyl-O-CH₂-.

270. Use according to claim 268 or 269, wherein R²⁷ is hydrogen.

271. Use according to claim 268 or 269, wherein R²⁷ is C₁₋₆-alkyl-O-CH₂-.

272. Use according to claim 271, wherein R²⁷ is H₃C-CH₂-O-CH₂-.

273. Use according to any of claims 268 to 272, wherein R²⁸ and R²⁹ independently of each other are hydrogen, halogen, dicyanovinyl, cyano, nitro, dinitrovinyl, alkyl optionally substituted with halogen, or aryl optionally substituted with one or more substituents selected from the group consisting of halogen, dicyanovinyl, cyano, nitro, and dinitrovinyl.

274. Use according to claim 273, wherein R^{28} and R^{29} independently of each other are halogen, cyano, C_{1-6} -alkyl optionally substituted with halogen, or aryl optionally substituted with one or more substituents selected from the group consisting of halogen, dicyanovinyl, cyano, nitro, and dinitrovinyl.

5

275. Use according to claim 274, wherein at least one of R^{28} and R^{29} is C_{1-6} -alkyl substituted with halogen.

276. Use according to claim 275, wherein at least one of R^{28} and R^{29} is trifluoromethyl.

10

277. Use according to claim 274, wherein at least one of R^{28} and R^{29} is C_{1-6} -alkyl.

278. Use according to claim 277, wherein at least one of R^{28} and R^{29} is ethyl or methyl.

15

279. Use according to claim 274, wherein at least one of R^{28} and R^{29} is halogen.

280. Use according to claim 274, wherein at least one of R^{28} and R^{29} is cyano.

20

281. Use according to claim 274, wherein at least one of R^{28} and R^{29} is aryl optionally substituted with one or more substituents selected from the group consisting of halogen, dicyanovinyl, cyano, nitro, and dinitrovinyl.

25

282. Use according to claim 281, wherein at least one of R^{28} and R^{29} is phenyl optionally substituted with one or more substituents selected from the group consisting of halogen, dicyanovinyl, cyano, nitro, and dinitrovinyl.

30

283. Use according to any of claims 268 to 272, wherein R^{28} and R^{29} together forms a benzene ring optionally substituted with one or more substituents selected from the group consisting of halogen, C_{1-6} -alkyl, dicyanovinyl, cyano, nitro, and dinitrovinyl.

284. Use according to claim 283, wherein R^{28} and R^{29} together forms a benzene ring.

35

285. Use according to claim 283, wherein R^{28} and R^{29} together forms a benzene ring substituted with one or more substituents selected from the group consisting of halogen, C_{1-6} -alkyl, dicyanovinyl, cyano, nitro, and dinitrovinyl.

286. Use according to any of claims 268 to 282, wherein R^{30} and R^{31} together forms a benzene ring optionally substituted with one or more substituents selected from the group consisting of halogen, C_{1-6} -alkyl, dicyanovinyl, cyano, nitro, and dinitrovinyl.

5

287. Use according to claim 286, wherein R^{30} and R^{31} together forms a benzene ring.

288. Use according to claim 286, wherein R^{30} and R^{31} together forms a benzene ring substituted with one or more substituents selected from the group consisting of halogen, C_{1-6} -alkyl, dicyanovinyl, cyano, nitro, and dinitrovinyl.

10

289. Use according to any of claims 268 to 285, wherein R^{30} is alkyl optionally substituted with halogen, or aryl optionally substituted with one or more substituents selected from the group consisting of halogen, dicyanovinyl, cyano, nitro, and dinitrovinyl.

15

290. Use according to claim 289, wherein R^{30} is alkyl optionally substituted with halogen.

291. Use according to claim 290, wherein R^{30} is C_{1-6} -alkyl optionally substituted with halogen.

20

292. Use according to claim 291, wherein R^{30} is methyl.

293. Use according to claim 291, wherein R^{30} is trifluoromethyl.

25

294. Use according to claim 289, wherein R^{30} is aryl optionally substituted with one or more substituents selected from the group consisting of halogen, dicyanovinyl, cyano, nitro, and dinitrovinyl.

30

295. Use according to claim 294, wherein R^{30} is phenyl optionally substituted with one or more substituents selected from the group consisting of halogen, dicyanovinyl, cyano, nitro, and dinitrovinyl.

296. Use according to any of claims 268 to 293, wherein R^{31} is hydrogen, dicyanovinyl, cyano, dinitrovinyl, or alkyl optionally substituted with halogen.

297. Use according to claim 296, wherein R^{31} is hydrogen, dicyanovinyl, cyano, dinitrovinyl, or C_{1-8} -alkyl optionally substituted with halogen.

298. Use according to claim 297, wherein R^{31} is hydrogen, dicyanovinyl, cyano, dinitrovinyl, or methyl.

299. Use according to claim 268, wherein the compound is
2-[[2-(4-chlorophenyl)-1H-indol-3-yl]methylene]malononitrile,
2-(4-chlorophenyl)-indole,

2,3-dimethyl-5-cyano-7-ethylindole, or

4-bromo-2-(4-chlorophenyl)-1-ethoxymethyl-5-trifluoromethyl-1H-pyrrole-3-carbonitril.

300. Use according to any of claims 1 to 13, wherein the compound has an E_{max} in Assay (I), as described in the specification, of less than 75% of the E_{max} of 2,4-dinitrophenol in Assay (I).

301. Use according to any of claims 1 to 26 or claim 300, wherein the compound has a slope calculated from the equation

$$X^n = (Y_2 - Y_0) / (Y_1 - Y_0)$$

wherein

Y_0 is the degree of stimulation measured as counts per minute (cpm) in Assay (I) in control samples without added test compound,

and

Y_1 is the degree of stimulation measured as cpm in Assay (I) with added test compound in a concentration of $EC_{50}/2$,

Y_2 is the degree of stimulation measured as cpm in Assay (I) with added test compound in concentration of $2 \times EC_{50}$, and

X is 2,

or

Y_1 is the degree of stimulation measured as cpm in Assay (I) with added test compound in a concentration of $EC_{50}/3$,

Y_2 is the degree of stimulation measured as cpm in Assay (I) with added test compound in concentration of $3 \times EC_{50}$, and

X is 3,

and

n is the slope,
equal to or less than the slope calculated with carbonylcyanide *p*-trifluoromethoxy-phenylhydrazone as test compound in Assay (I).

- 5 302. Use according to any of claims 1 to 39, or any of claims 300 to 301, for which the value for the glucose utilisation caused by the compound in Assay (I) in concentrations of from CE_{max} to ten times CE_{max} does not fall significantly below the value of E_{max} for the compound in Assay (I),
- 10 303. Use according to any of claims 300 to 302, wherein the compound is a chemical uncoupler as defined in Assay (II), as described in the specification.
304. Use according to any of claims 300 to 303, wherein the compound is a cation.

ABSTRACT

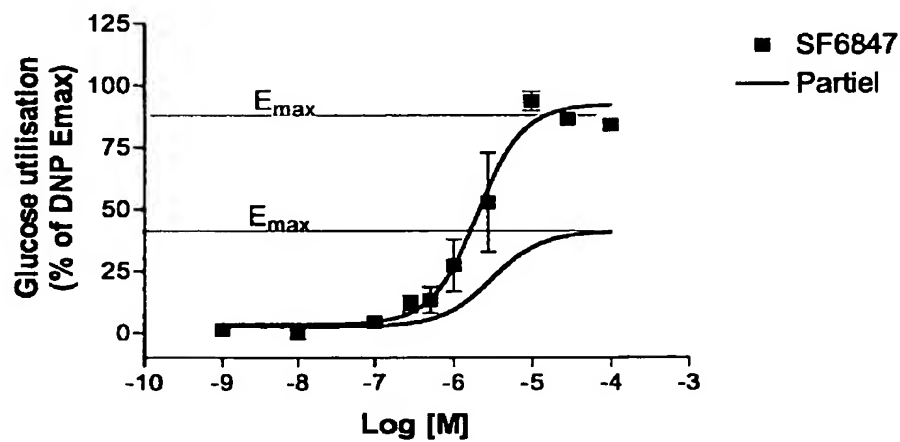
This invention relates to chemical uncouplers with a broader safety window making the use of them in treating obesity and, consequently, in the treatment of obesity related diseases and conditions such as atherosclerosis, hypertension, diabetes, especially type 2 diabetes (NIDDM (non-insulin dependent diabetes mellitus)), impaired glucose tolerance, dyslipidemia, coronary heart disease, gallbladder disease, osteoarthritis and various types of cancer such as endometrial, breast, prostate and colon cancers and the risk for premature death as well as other conditions, such as diseases and disorders, which conditions are improved by an increase in mitochondrial respiration, more attractive.

04 JUNI 2003

Modtaget

FIGURE 1/3

Hep-G2 cells



04 JUNI 2003

Modtaget

FIGURE 2/3

Hep-G2 cells

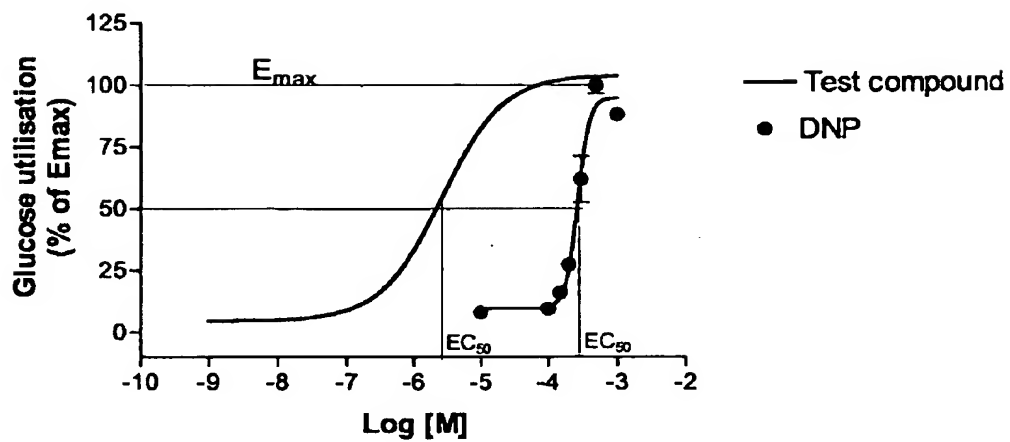


Figure 3/3

Hep-G2 cellsPatent- og
Varemærkestyrelsen

04 JUNI 2003

Modtaget

